Effect of NPK application on rooibos (*Aspalathus linearis*) under Clanwilliam field conditions

by

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DECLARATION

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ABSTRACT

Currently no macronutrient fertiliser recommendations have been established for rooibos plants under field conditions. The aim of this study was to examine the interactive effect of NPK (nitrogen, phosphorus, potassium) on young rooibos plants' growth and survival, and soil chemistry and fertiliser leaching under Clanwilliam field conditions with an aim to establishing soil and foliar nutrient norms for optimum rooibos production. A field trial was established at Vaalkrans Farm, Clanwilliam district in June 2016. Rooibos seedlings were fertilised at planting as a completely randomised design in combinations of various levels of N (0, 20, 40, 60 mg/kg) as NBPT-coated urea, P (0, 15, 30, 45, 60 mg/kg) as triple superphosphate and K (0, 20, 40, 60, 80 mg/kg) as potassium chloride (KCl). The N and K applications were split, 50% at planting and the remainder top-dressed after 2 months. The fertilisers and application rates were selected based on previous seedling greenhouse trials. Parameters measured during the trial included: soil pH, electrical conductivity (EC), total carbon (C) and N, mineral N, Bray II P, exchangeable cations, micronutrients, soil enzyme activity, plant height, survival, biomass, and foliar nutrient content. The movement of the applied fertilizer was also determined on selected treatments, and a pot trial to determine the effect of lime application of rooibos seedling growth was performed. Initially, during the wet winter months, the application of P at 15 and 30 mg/kg stimulated biomass production. However, after the dry summer it was observed that all P applications suppressed plant growth and decreased plant survival, and this effect was more pronounced as P application rate increased. Foliar P and shoot biomass were negatively correlated (R^2=0.5929). No interactive effect between N and P on biomass response was found, and N application could not help rooibos to overcome P-toxicity, contrary to previous studies. The highest above-ground biomass yields were recorded at K application rates of 20 – 40 mg/kg. When yield was adjusted according to mortality, the 20 mg/kg K treatment had the largest yield (597 kg/ha), nearly double that of the unfertilised control. Due to the low intensity rainfall experienced in Clanwilliam during the field trial, the fertiliser had not leached significantly in the soil profile, and the majority remained where it was initially placed at planting (20 – 30 cm) and on surface (0 – 20 cm). Rooibos seedling biomass responded positively to lime application at all rates up to an equivalent of 1.29 t/ha in a greenhouse pot trial. Application rates of 1 – 1.3 t/ha nearly doubled the mass of rooibos seedlings after two months. The ideal pH for rooibos seedling growth in this study was found to be around pH (KCl) 7.4. This study highlights the importance of field trials, as opposed to short-term greenhouse trials, as the effect of nutrients combined with climate can have deleterious effects. It is recommended that young rooibos plants do not receive any P fertilisers at planting, but receive up to 20 mg/kg of N and between 20 – 60 mg/kg of K (applied as split application).

OPSOMMING

Huidiglik is daar geen aanbevelings vir makrovoedingstof-bemesting vir rooibosplante onder veldtoestande vasgestel nie. Die doel van hierdie studie was om die interaktiewe effek van NPK (stikstof, fosfor en kalium) op jong rooibosplante se groei en oorlewing te bestudeer, asook grondchemie en bemestingloging onder Clanwilliam veldtoestande met die doel om grond- en blaarvoedingstof standaarde vas te stel vir optimale rooibos produksie. ‘n Veldproef is in Junie 2016 by Vaalkrans Plaas, Clanwilliam gestig. Rooibos-saailinge was tydens planting bemes as ‘n ewekansige ontwerp in kombinasies van verskeie vlakke van N (0, 20, 40, 60 mg/kg) as NBPT-bedekte urea, P (0, 15, 30, 45, 60 mg/kg) as trippel superfosfaat en K (0, 20, 40, 60 mg/kg) as kaliumchloried. Die N- en K-toedienings was opgedeel, 50% by die plant-proses en die oorblywende daarvan is na 2 maande oppervlakkig toegedien. Die bemestingstowwe en toedieningshoeveelhede is gekies gebaseer op vorige kweekhuis proewe op saailinge. Kriteria wat gedurende die proef gemeet was sluit die volgende in: grond pH, elektriese konduktiwiteit (EK), totale koolstof (C) en N, minerale N, Bray II P, uitruilbare katione, mikrovoedingstowwe, grondensiem-aktiwiteit, plant-hoogte, oorlewing, biomassa en blaarvoedingstof-inhoud. Die beweging van die toegediende bemestingstof was ook by geselekteerde behandelings vasgestel en ‘n potproef om die uitwerking van kalk-toediening op rooibos-saailinge se groei vas te stel is uitgevoer. Aanvanklik, gedurende die nat wintersmaande, het die toediening van P teen 15 en 30 mg/kg biomassa produksie gestimuleer. Na die droë somer was daar egter waargeneem dat al die P-toedienings plantegroei onderdruk het en plant-oorlewing verminder het, en hierdie uitwerking was duideliker soos wat die P-toedieningshoeveelheid toegeneem het. Blaar P en loot-biomassa het ‘n negatiewe verband met mekaar gehou (R^2=0.5929). In teenstelling met vorige studies, is geen interaktiewe uitwerking is gevind tussen N en P op biomassa-reaksie nie en N-toediening kon nie rooibos help om P-toksisiteit te oorwin nie. Die hoogste bogrondse biomassa-opbrengs was teen K-toedieningshoeveelhede van 20-40 mg/kg waargeneem. Toe die opbrengs aangepas was volgens die sterftesyfer het die 20 mg/kg K-toediening die hoogste opbrengs gehad (597 kg/ha), byna dubbeld wat die onbemeste kontrole was. As gevolg van lae-intensiteit reënval wat in Clanwilliam ervaar is tydens die veldproef het die bemestingstof nie aansienlik in die grondprofiel geloog nie en die meerderheid daarvan het agtergebly waar dit oorspronklik geplaas is tydens die plant-proses (20 – 30 cm) en op die oppervlak (0 – 20 cm). Rooibos-saailing biomassa het positief gereageer op kalk-toediening teen alle hoeveelhede tot en met ‘n ekwivalent van 1.29 t/ha in ‘n kweekhuis potproef. Toedieningshoeveelhede van 1 – 1.3 t/ha het die massa van rooibos-saailinge naastenby verdubbel na twee maande. Die ideale pH vir rooibos-saailinge se groei in hierdie studie was bevind om by pH (KCl) 7.4 te wees. Hierdie studie beklemtoon die belangrikheid van

veldproewe, in teenstelling met korttermyn kweekhuis-proewe, aangesien die uitwerking van voedingstowwe in samewerking met die klimaat nadelige nagevolge kan hê. Dit word aangeraai dat jong rooibosplante geen P-bemesting ontvang tydens planting nie, maar wel dat 20 mg/kg N en tussen 20 – 60 mg/kg K toegedien word as gesplete toedienings.

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CHAPTER 1 – General introduction and research aims

Farmers report that *Aspalathus linearis* (rooibos tea) plantation yields tend to decrease five years after the establishment of a new field on virgin fynbos lands. This decrease is most likely due to the depletion of nutrients essential to plant growth, as fertilisation and liming are not common production practices in the rooibos industry. Little research has been carried out on the cultivation of rooibos, and the majority of studies examining nutrient uptake and interaction have been greenhouse pot trials. The results of these trials cannot be translated into fertiliser recommendations since the rooibos plants in these trials were grown under controlled conditions, and are therefore of little practical use to the rooibos farmer or to the rooibos tea industry. Therefore, the aim of Chapter 3 in this dissertation was to investigate the interactive effects of the application of the macronutrients nitrogen (N), phosphorus (P), and potassium (K) on young rooibos plant growth, survival and nutrient uptake, its N-fixation efficiency, above-ground biomass yields, soil chemistry and selected soil microbial enzymes under field conditions. In Chapter 4, the distribution and movement of moderate amounts of applied N, P and K was studied in order to determine the movement of solutes between planting in June and the end of the rainfall season in October, to optimise fertiliser application. The soil parameters examined in this solute movement were pH, electrical conductivity (EC), mineral ammonium and nitrate, and Bray II extractable P and K. Analysis of the NPK content of the seedlings at planting in June and of plants in both unfertilised and moderately fertilised treatments in October was done to determine the effect of fertilisation. As little is known about the ideal soil pH levels for the cultivation of rooibos, the aim of Chapter 5 was to perform a lime incubation study and a two-month pot trial to observe the effect of lime application on pH, EC, exchangeable acidity and calcium, the biomass response of rooibos seedlings, and to provide initial data for the establishment of future field trials. This dissertation aims improve the understanding of rooibos nutrient requirements and to provide guidelines for macronutrient fertiliser recommendations for young rooibos plants in field cultivation in the Clanwilliam region. This information can be applied to better manage soil fertility in rooibos tea production, improving yields without compromising soil quality and the sustainability of the rooibos industry.

CHAPTER 2 – Literature review

2.1. Introduction

The majority of scientific research on *Aspalathus linearis* (rooibos tea) has focussed on the medicinal benefits of the plant and optimising tea quality. Of the little research that has been carried out on the optimal soil nutrient levels for optimum growth of rooibos, the majority have been greenhouse or hydroponic studies. The aim of this literature review is to discuss the botanical and geographical context of rooibos, describe the general cultivation practices carried out in the production of this crop and to supply an overview of previous research done on its nutrient requirements, highlighting remaining gaps in knowledge.

2.2. Classification, description and domestication.

Aspalathus linearis (hereafter also referred to as "rooibos" or the "rooibos plant") is a leguminous shrub native to the fynbos biome in the Western and Northern Cape Provinces of South Africa (Van Der Bank et al. 1995). The term "rooibos" refers to both the plant and the herbal tea produced from it (Hawkins et al., 2011). The botanist Carl Thunberg was the first to record its harvest and use by rural people in the Cederberg region in 1772 (Morton 1983). *Aspalathus linearis* is a highly polymorphic species complex comprised of several ecotypes and possible subspecies (Hawkins et al., 2011; Malgas et al., 2010). (Dahlgren, 1968) first described the various forms and classified the species complex. Rooibos has also been classified into several ecotypes according to growth form, and genetic makeup (Malgas et al., 2010; Archer et al., 2008), while seven types were distinguished according to tea colour and flavonoid content by (Heerden et al., 2006). From a practical perspective the species is considered to take four forms: *Rooitee*, *vaaltee*, *swarttee* and *rooibruintee*, referring to the red, grey, black and red-brown colour of the tea, respectively. *Rooitee* is divided into a further two types, *Nortier* and *Cederberg*, of which the former is the cultivated form, selected from naturally occurring populations of the latter (Smith, 2014). The *Nortier* type originated in the northern Cederberg region (Van Der Bank et al., 1995; Hawkins et al., 2011) and was selected in the Pakhuis Mountains in the 1920's by Dr Pieter le Frais Nortier and local Khoi people based on growth rate, seed production and most importantly, taste. The other types (*vaaltee*, *swarttee* and *rooibruintee*) produce a tea with disagreeable flavour (Cheney & Scholtz, 1963). Rooibos is one of the few fynbos plants to have undergone domestication (Van Der Bank et al., 1995) and is also a young crop, having been cultivated for less than century. (Hawkins et al., 2011) suggest that the use of only one selection and the implication of low genetic diversity risks the possibility of widespread crop failure, especially due to increasing pest problems and drought pressure.

Rooibos is grown in the Western and Northern Cape provinces of South Africa. Most rooibos is grown around the town of Clanwilliam in six main production zones (Citrusdalberg, Eendekuil, Nardouwsberg/Agterpakhuis, Seekoevlei and Vanrhynsdorp (Smith, 2014). Rooibos cultivation is rainfed, under a semiarid climate with a strong seasonal rainfall pattern: rain falls during winter, while rooibos grows in summer during the period of most aridity and evaporative demand. Besides the summer water deficit, the low nutritional status of the soil is also a factor limiting biomass production (Lotter et al., 2014). The area of cultivation is limited by climate to regions with rainfall of 250-300 mm per year ((Lotter et al., 2014); van Putten as cited by Smith, 2014) and environmental legislation which prevents the ploughing of virgin land (Smith, 2014). A crop cycle lasts 3-5 years before the rooibos plants begin to die and yield declines to an unacceptable level (Smith, 2014). The reason for this high mortality rate is unknown, but according to a study by Cocks & Stock (2001) it is likely biological and not environmental. This opinion is substantiated by the role of *Aspalathus* species as post-fire colonisers. The plants are ploughed up and a cover crop of wheat or oats is sown for one or two years before rooibos is replanted. Rock phosphate is sometimes applied to the land before planting this cover crop (Joubert et al., 1987) and this may be the only form of nutrient input in the crop system, as rooibos is rarely fertilised. The cover crop serves to break pathogen life cycles and prevent the erosion that would occur if the field were bare-fallowed (Smith, 2014). The soil is ripped at the start of each crop cycle, just before planting.

2.3. General soil conditions under cultivation

The soils on which rooibos is cultivated are generally nutrient-poor leached soils, characterised by low cation exchange capacity (CEC) due to low organic matter and clay content, and high acidity (Muofhe & Dakora, 1999a). Plant growth is further restricted by the shortage of plant-available macro- and micronutrients. Major sources of nitrogen (N) in fynbos soils are ultimately of atmospheric origin, fixed by legumes such as *Aspalathus* in symbiosis with Rhizobia, and cycled in the form of plant material (Herppich et al., 2002).

2.4. Adaptations to low soil nutrient levels

The occurrence of cluster roots on *A. linearis* was first recorded by (Hawkins et al., 2011) and was again observed by (Smith, 2014). Their formation is associated with P levels, and have been shown to grow on five-month old seedlings in solution culture when the phosphorus supply was decreased relative to nitrogen supply (Maistry et al., 2015). Rooibos forms symbiotic relationships with *Bradyrhizobium*, allowing it to satisfy most of its N requirements by means of N fixation. More than 70% of N obtained can be obtained in this way. *A. linearis* plant material has higher N content than that surrounding non-fixing vegetation. Rhizobia in root nodules fix approximately 105 – 128 kg of N per hectare (Muofhe & Dakora, 1999a;

Maistry et al., 2013). In this sense, *Aspalathus spp.* seem to have an ecological function similar to *Cyclopia spp*. (Spriggs & Dakora, 2009).

Other adaptations that enable it to survive in harsh environments include schlerophylly and modification of rhizosphere pH by extruding HCO_3^-, OH^- and organic acids (Dakora & Phillips, 2002) to facilitate the uptake of limiting nutrients such as P. This latter adaptation is was only observed on 2 – 4 year old plants; rhizosphere pH did not differ significantly from non-rhizosphere pH in one-year-old plants (Muofhe & Dakora, 2000). The rooibos plant also “pools” nutrients: For example, P is accumulated and stored in the plant during winter prior to period of grand growth, when it is made available for the spring growth flush. Overall, there is still a lack of knowledge regarding the response of the rooibos plant to in-field nutrition and water relations (Lotter et al., 2014).

2.5. Rooibos nutrient trials

A pot trial by Joubert et al. (1987) was the first and remains the only study to have suggested fertiliser norms for the optimum dry matter yield of rooibos seedlings. Optimum growth was observed at levels of 15-20 mg/kg Bray II plant-available P, while higher concentrations were found to decrease biomass yield significantly. In comparing the effects of different P-fertilisers, the study also found that source of P is inconsequential as long as it does not contain large amounts of magnesium (Mg). While the other nutrients were studied individually, the authors studied the combined effect of Mg and N. Due to the interaction between Mg and N at high (>30 mg/kg) Mg content, the response to applied N is dramatically reduced in terms of biomass yield. Therefore, it was recommended that the fertilisation of rooibos with magnesium-containing nutrients be avoided. Furthermore, the uptake of N, P, K and Ca was seen to be reduced when Mg occupies 10 % or more of cation-exchange capacity. Ideal growth response was obtained when at an application rate of 60 mg/kg Bray II K was used, and Ca in the form of lime sufficient to adjust the pH ($CaCl_2$) to 5.0 was added. Application rates of 10-15 mg/kg of N in the form of ammonium nitrate yielded the highest amount of dry matter, but Joubert et al. (1987) are of the opinion that the application of N is unnecessary as the rooibos plant is capable of fixing its own N. This stands in contrast to later research (Muofhe & Dakora, 1999) that demonstrated that the application of N is useful in increasing biomass yields. It must be emphasised that these norms are based on a pot trial, and may be difficult to translate to norms for field cultivation.

In a study on one-year-old rooibos plants removed from the field and then cultivated in a greenhouse pot trial, (Muofhe & Dakora, 1999a) found that the split application of N, Ca and P fertilisers to the plants significantly increased mass compared to unfertilised control, contradicting findings by the same authors that Ca suppressed rooibos seedling growth (Muofhe and Dakora, 1999b). An increase in root dry matter contributed more to this effect than that which was in the shoots. This difference in the allocation of total dry matter was most pronounced in 2 – 3 year old plants. Nitrogen allocation to the below-ground parts of the plant was found to increase similarly. Phosphate-supplied plants had lower delta N-15 ($\delta^{15}N$) values than control and Ca-supplied plants. This suggests that P-supply plays an important role in the N fixation process. Nitrogen fixation was found to increase by 4-85% with an increase in nutrient supply. Total, shoot and root N content increased significantly as the plant aged from one to three years old. The percentage of N derived from fixation and the total amount fixed per plant also increased, and that which was fixed by 3-year-old plants differed significantly from that of the younger plants, although total dry matter did not increase significantly over the same period. Both of the (Muofhe & Dakora, 1999a, 1999b) studies lack soil analysis data, and in both studies the K was added in the form of potassium phosphate, preventing the effects of the K and phosphate to be assessed individually. Nevertheless, their findings challenge the notion that the growth of the rooibos plant (and that of fynbos plants in general) is limited genetically, and suggest P may be the most limiting nutrient for rooibos growth in Clanwilliam soil.

Maistry et al. (2015) performed a five-month study on the response of rooibos seedlings grown in sand substrate and in nutrient solutions to various combinations of N and P levels. They found that low P levels suppressed biomass accumulation, and increased the proportion biomass in the form of cluster roots, while higher levels suppressed their formation. Higher N:P ratios also stimulated P-acquisition mechanisms such as the formation of cluster roots. At a P supply of 100 µM, shoot P concentration decreased nearly threefold as N supply was increased from 100 to 700 µM. At both high P (100 µM) and high N (700 µM), the root:shoot dry matter ratio decreased while total dry matter yield increased. The authors conclude that the poor performance of unfertilised rooibos plants is not necessarily due to low P, but an oversupply of N which raises the N:P ratio.

In a study on organic cultivation of rooibos in Nieuwoudtville, Chimphango et al. (2016) found soil C in rooibos fields to be positively correlated with soil Ca, Mg, K and Na, although no correlation with P or pH was observed. The same study found no correlation between soil fertility and age of the cultivated plots, over a five-year trial period. The authors hypothesise that cultivation practises such as mulching, the planting of hedgerows to limit wind erosion and the sowing of cover crops. However, the trial may not have been long enough to observe a statistically significant decline in soil fertility. The soil conservation methods mentioned can only slow down nutrient loss. Nutrient removal may be offset by N-fixation, but nutrients such as P and K will inevitably be depleted through the annual removal of plant matter.

(Smith, 2014) studied the effect of rooibos cultivation on soil quality compared to uncultivated fynbos soils. An increase in soil P with cultivation was found, probably due to fertilisation of cover crops with rock phosphate. High P levels supressed plant growth, lowered tea quality and inhibited the mycorrhizal colonization of plant roots. The same effect was observed during a trial in which high P compost was applied (Smith, 2014). He found that with cultivation, soil C and basic cations such as Mg, Ca and Na decreased.

2.6. Concluding remarks

A field trial which will study the interaction between macronutrients on rooibos plant growth and tea quality under field conditions has not yet been done. There are still large gaps in our knowledge regarding the soil aspects of rooibos cultivation for efficient commercial production, such as macro- and micronutrient requirements and plant-water relations.

CHAPTER 3 – Field trial

3.1. Introduction

4. Little research has been carried out on the cultivation of *Aspalathus linearis*. The majority of studies examining nutrient uptake and interaction have been greenhouse pot trials. The results of these trials cannot be translated into fertiliser recommendations since the rooibos plants in these trials were grown under controlled conditions, and are therefore of little practical use to the rooibos farmer or to the rooibos tea industry. The aim of this study is therefore to investigate the interactive effects of the application of the macronutrients N, P, and K on young rooibos plant growth, survival and nutrient uptake, its N-fixation efficiency, above-ground biomass yields, soil chemistry and selected soil microbial enzymes under field conditions in the Clanwilliam area. The study was performed in two parts: an N × P combined factorial, as previous studies have emphasised the importance of these two elements and their interaction with regards to rooibos growth (Maistry et al., 2015), and a K × NP experiment, in which K was applied with and without moderate amounts of N and P, in the event that K may have been a limiting nutrient. This study intends to provide guidelines for macronutrient fertiliser recommendations for young rooibos plants in field cultivation. This information can be applied to better manage soil fertility in rooibos tea production, improving yields without compromising soil quality and the sustainability of the rooibos industry.

4.1. Methods and materials

4.1.1. Experimental design

In March 2016, a 3 ha fallow field on the farm Vaalkrans, Clanwilliam, Western Cape, South Africa (GPS coordinates: 33 00 38.07S, 18 55 21.47E) was selected for the trial (Figure 3.1). The site was selected due to its inherent low nutrient content which was necessary for a fertiliser trial. The soil on the trial site is representative of those on which rooibos is commonly cultivated in the region, being coarse, sandy soils derived from quartzitic sandstone.

Figure 3.1. Aerial image of trial site at the farm Vaalkrans, Clanwilliam.

A soil depth map was created by surveying the site on a 10 × 10 m grid using an auger and a GPS device to plot a map of soil depth on QGIS software (see Figure 3.2). Areas of similar, moderate soil depth (0.40 – 1.0 m) were selected for an experiment examining the interactive effect of NPK fertilisation, and deeper (≥1m) areas were selected for an experiment examining the effect of depth on fertiliser movement.

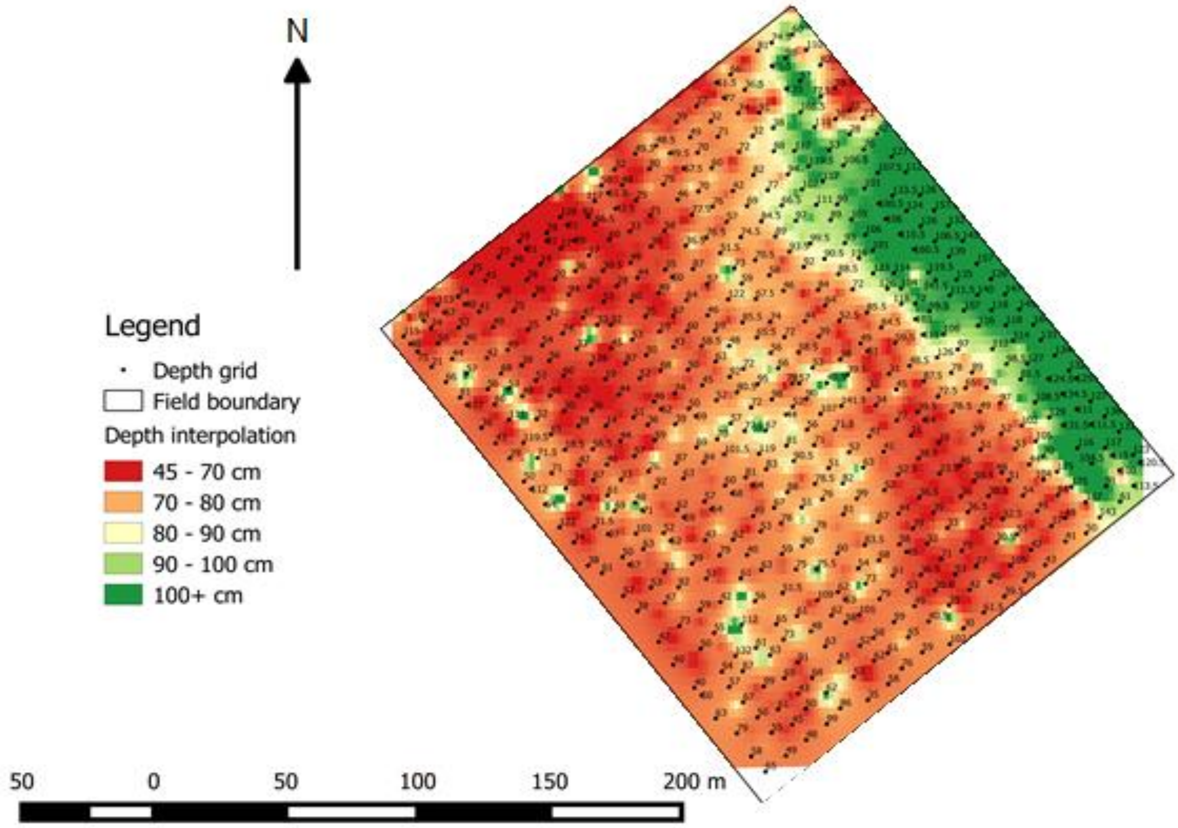

Figure 3.2. Soil depth map at the field site (surveyed on a 10 x 10 m grid) on Vaalkrans Farm.

The experimental design was laid out in a completely randomised design on the areas of the field with moderate to deep (> 0.40 m) soil depth (Figure 3.2). Experimental blocks for treatment repetitions with an area of 81 m^2 were laid out which each contained 72 rooibos seedlings. Each block consisted of 6 rows of 12 seedlings planted 0.75 m apart with a row spacing of 1.5 m. The field trial was divided in two sections: an experiment to study the interactive effect of N versus P application, and one to study the interactive effect of K versus N and P application. Fertiliser application rates used were 0, 20, 40 and 60 mg/kg N in all combinations with 0, 15, 30, 45, and 60 mg/kg P for the N×P experiment and 0, 20, 40, 60, 80 mg/kg K with and without 20 mg/kg N and 30 mg/kg P for the K × NP experiment (Table 3.1 and Table 3.2). These application rates were chosen based on the recommendations of the rooibos seedling pot trial study of Joubert et al (1987). Higher application rate treatments (mg/kg) were included compared to those used in the aforementioned study, since in field trials there is a greater loss of applied nutrients than in confined pot trials. Furthermore, previous studies also indicated that there are beneficial effects of adding N as it helps P sensitive crops overcome P toxicity (Maistry et al., 2013; Maistry et al., 2015). Superimposed on the design layout, ten blocks of 1 215 – 1 620 m^2 were used to delineate sampling areas for baseline soil analysis.

Table 3.1. List of treatments for the N × P experiment.

Treatment	N (mg/kg)	P (mg/kg)
1	0	0
2	0	15
3	0	30
4	0	45
5	0	60
6	20	0
7	20	15
8	20	30
9	20	45
10	20	60
11	40	0
12	40	15
13	40	30
14	40	45
15	40	60
16	60	0
17	60	15
18	60	30
19	60	45
20	60	60

Table 3.2. List of treatments for the K × NP experiment.

Treatment	N (mg/kg)	P (mg/kg)	K (mg/kg)
1	0	0	0
21	0	0	20
22	0	0	40
23	0	0	60
24	0	0	80
8	20	30	0
25	20	30	20
26	20	30	40
27	20	30	60
28	20	30	80

The NPK fertilisers used in the trial were provided by Yara Cape Ltd. in Paarl. The selected N fertiliser was Yara Vera^{TM} AmiPLUS, which is a coated urea product containing the urease enzyme inhibitor NBPT. This inhibitor slows the conversion of urea to mineral ammonium, greatly reducing volatilisation losses and improving N use efficiency by plants. The P fertiliser used was Yara Maxiphos 20 P (triple superphosphate (TSP) – $Ca(H_2PO_4)_2$) and the K fertiliser used was Yara potassium chloride. These nutrient sources were selected as they contain very readily available forms of the macronutrients and previous studies had demonstrated their effectiveness on rooibos plants (Joubert et al., 1987). The application rates (kg/ha) given Table 3.3 and Table 3.4 and are based on a bulk density of 1600 kg/m^3, which is typical of the sandy soils on the farm according to Smith (2014), an application band width of 0.4 m and an incorporation depth of 0.2 m. The field trial was initiated on 16 June 2016 when the first fertiliser treatments were applied and the rooibos seedlings were planted. The fertiliser treatments were applied by hand to the planting row (approximately 0.4 m width) and ploughed into the soil to a depth of approximated 0.2 m using a shallow tine implement and subsequent mixing by the tractor wheels as they passed over the soil twice while ploughing, going in opposite directions each time. All of the P fertiliser treatments were applied at planting, whereas, only 50% of the N and K fertiliser treatments were applied at planting. Directly after application of the fertiliser treatments, the 5-month old seedlings which been sown in a nursery four months previously were planted by hand by farm workers in the rows (routine practice). The remainder of the N and K fertiliser was applied by hand two months later (18 August 2016) in the planting row as a top dressing.

Table 3.3. Fertiliser application rates for urea and triple superphosphate used in the N × P experiment.

	0 mg/kg N (0 kg/ha N)	20 mg/kg N (13.8 kg/ha N)	40 mg/kg N (27.6 kg/ha N)	60 mg/kg (41.1 kg/ha N)
0 P mg/kg P (0 kg/ha P)	0 urea 0 TSP	30 kg/ha urea 0 TSP	60 kg/ha urea 0 TSP	90 kg/ha urea 0 TSP
15 mg/kg P (10.4 kg/ha P) (6.8 kg/ha Ca)	0 urea 52 kg/ha TSP	30 kg/ha urea 52 kg/ha TSP	60 kg/ha urea 52 kg/ha TSP	90 kg/ha urea 52 kg/ha TSP
30 mg/kg P (20.7 kg/ha P) (13.5 kg/ha Ca)	0 urea 104 kg/ha TSP	30 kg/ha urea 104 kg/ha TSP	60 kg/ha urea 104 kg/ha TSP	90 kg/ha urea 104 kg/ha TSP
45 mg/kg P (31.2 kg/ha P) (20.3 kg/ha Ca)	0 urea 156 kg/ha TSP	30 kg/ha urea 156 kg/ha TSP	60 kg/ha urea 156 kg/ha TSP	90 kg/ha urea 156 kg/ha TSP
60 mg/kg P (41.4 kg/ha P) (27.0 kg/ha Ca)	0 urea 208 kg/ha TSP	30 kg/ha urea 208 kg/ha TSP	60 kg/ha urea 208 kg/ha TSP	90 kg/ha urea 208 kg/ha TSP

Table 3.4. Fertiliser application for urea, triple superphosphate and KCl used in the K × NP experiment.

	0 N 0 P	20 mg/kg N and 30 mg/kg P
0 mg/kg K (0 kg/ha K)	0 urea 0 TSP 0 kg/ha KCl	30 kg/ha urea 104 kg/ha TSP 0 kg/ha KCl
20 mg/kg K (13.9 kg/ha K)	0 urea 0 TSP 27.7 kg/ha KCl	30 kg/ha urea 104 kg/ha TSP 27.7 kg/ha KCl
40 mg/kg K (27.7 kg/ha K)	0 urea 0 TSP 55.5 kg/ha KCl	30 kg/ha urea 104 kg/ha TSP 55.5 kg/ha KCl
60 mg/kg K (41.6 kg/ha K)	0 urea 0 TSP 83.2 kg/ha KCl	30 kg/ha urea 104 kg/ha TSP 83.2 kg/ha KCl
80 mg/kg K (55.5 kg/ha K)	0 urea 0 TSP 110.9 kg/ha KCl	30 kg/ha urea 104 kg/ha TSP 110.9 kg/ha KCl

4.1.2. Soil sampling

4.1.2.1. Preliminary sampling

Prior to establishment of the field trial, composite (6) soil samples for baseline soil analysis were taken in ten large blocks augered at 0 – 20 cm and 20 – 40 cm.

4.1.2.2. Field trial sampling

Soil samples were taken from all treatments at the termination of the field trial one year after planting after the first rainfall, in May 2017. The samples were taken with a soil auger at 0 –

20 cm and 20 – 40 cm in each replicate, 0.2 m from the base of a living rooibos plant and in the row. Composite samples (6) were made for each treatment replicate. The soil samples were then air dried for subsequent analysis.

4.1.3. Plant sampling

4.1.3.1. Plant height

Vigour was assessed by measuring the height of the rooibos plants from the soil line to the tip of the tallest shoot at four, eight and twelve months (October 2016, February 2016 and May 2017) after planting. Plant height was measured on 6 randomly selected plants from each replicate on all treatments.

4.1.3.2. Plant survival

The number living plants were counted in each replicate eight and twelve months after planting (February and May 2017). Plant survival was expressed as the percentage of surviving plants remaining of the 72 planted in each replicate.

4.1.3.3. Above-ground biomass

The above-ground growth of the six plants used for plant height in each treatment replicate was destructively harvested at the soil line 12 months after planting. The plant samples were air-dried, the mass was determined and the mean above-ground mass per plant was calculated.

4.1.4. Rhizosphere soil enzyme sampling

Rhizosphere soil was collected on the 31st of May 2017 at 0 – 20 cm depth from three random plants in each replicate (4) of selected treatments: control, 15 mg/kg P, 20 mg/kg N and 20 mg/kg N, 15 mg/kg P. Rhizosphere soil samples were immediately placed in liquid nitrogen until enzyme extraction was performed.

4.1.5. Soil analyses

4.1.5.1. Soil texture and bulk density

Textural analysis was done the using the American Society for Testing and Standards (ASTM) D6913 standard and bulk density was determined by undisturbed core method (Blake & Hartge, 1986).

4.1.5.2. Soil pH and EC

Soil pH was measured in distilled water and in a 1M KCl solution in a 1:2.5 solid to liquid ratio (Rowell, 1994).

Soil electrical conductivity was measured in a 1:2.5 water extract and converted to the equivalent for a saturated paste extract (Sonmez et al., 2008).

4.1.5.3. Total C and N

Soil samples were ball-milled, and the total C and N was determined by dry combustion using a EuroVector EA Elemental analyser.

4.1.5.4. Plant-available P

The Bray II method was used to determine the level of P readily available for plant uptake. This method is commonly used for P determination on acid soils (Kuo, 1996).

4.1.5.5. Exchangeable cations and acidity

Exchangeable Ca, Mg, K and Na was determined via the 1 M ammonium acetate (pH 7.0) method, using the centrifuge procedure. Reliable results for Ca^{2+} content can be expected as the soil in question is acidic and lacks free carbonates. Exchangeable acidity was measured using the 1 M KCl extraction method (Thomas, 1982)

4.1.5.6. Plant-available micronutrients

The di-ammonium EDTA method was used for the determination of plant-available manganese, iron, copper and zinc (Olsen & Ellis, 1982).

4.1.6. Soil enzyme assays

4.1.6.1. Enzyme extraction

The soil samples were ground in liquid nitrogen and 500 mg of each sample was extracted with 2 mL extraction buffer, vortexed and then centrifuged at 3500g at 2°C for 10 minutes. The solid phase was discarded and the resultant supernatant was then centrifuged again at 30 000g at 2°C for 20 minutes. The solid phase was again discarded and the supernatant kept on ice for further determinations.

4.1.6.2. Preparation of standards

A Bradford Standard Curve was created for protein concentration determination using the spectrophotometric method (Thuynsma et al., 2014 ab).

4.1.6.3. XDH

XDH assays were done according to the method of (Khadri et al., 2001) and read at 340 nm for five minutes (Magadlela et al., 2016, 2017).

4.1.6.4. GOGAT

Assay mixture was mixed without adding 2-oxoglutarate and L-glutamine. Assays were done according to the method outlined in Feng-Ling & Cullimore (1988), and read continuously at 340 nm for 5 minutes at 18 – 22ºC (Magadlela et al., 2016, 2017).

4.1.6.5. PK

Four blanks were made: distilled water, assay mixture without ADP and PEP, without ADP and without PEP. Crude supernatant (30 µL) was added to 220 µL to start the reaction and read continuously at 340 nm for 5 minutes (Thuynsma et al., 2014 ab).

4.1.6.6. ME

Two blanks were made with 250 µL ultra-pure distilled water, and 250 µL of assay mixture without malic acid. Assay mixture (220 µL) was added to 30 µL of supernatant, and read continuously at 340 nm for 5 minutes (Thuynsma et al., 2014 ab).

3.1.1.6 MDH

Two blanks were made with 250 µL ultra-pure distilled water, and 250 µL of assay mixture without oxaloacetic acid (OAA). Assay mixture (220 µL) was added to 30 µL of supernatant, and read continuously at 340 nm for 5 minutes.

4.1.7. Statistical analysis

STATISTICA 12 data analysis software was used to perfom statistical analysis of soil, foliar and yield data. Multivariate tests of significance were performed on each soil, foliar, yield and enzyme parameter. Where no significant interactions between treatments were observed, univariate tests of significance were then performed. Least significant difference (LSD) tests were used to separate differences between treatment means. Spearman and Pearson tests were used to determine correlations between soil and foliar, soil and yield and foliar and yield parameter.

3.3. Results and discussion

3.3.1. Preliminary analysis

The average soil pH (H_2O) values at the field site were acid (pH 4.8 in the topsoil and 4.6 in the subsoil) which is typical for rooibos production areas (Smith, 2014). The topsoil C and N was very low at 0.25 % C and 0.02 % N. The Bray II P was also very low at around 4 mg/kg in the topsoil and 2.2 mg/kg in the subsoil. The soil effective cation exchange capacity (ECEC) was also very low (less than 1 $cmol_c$/kg) which is expected for such coarse sandy soils with low C (Table 3.5).

Table 3.5. Average soil chemical properties at the field trial site prior to establishment of the trial.

Soil depth (cm)	pH (H_20)	pH (KCl)	EC (µS/cm)	% C	% N	Bray II P (mg/kg)	Ca (cmolc/kg)	Mg (cmolc/kg)	Na (cmolc/kg)	K (cmolc/kg)	Total KCl Exch. Acidity (cmolc/kg)	ECEC (cmolc/kg)	Cu (mg/kg)	Zn (mg/kg)	Mn (mg/kg)
0 - 20	4.81	4.22	11.6	0.25	0.02	4.00	0.27	0.08	0.04	0.21	0.16	0.76	0.32	0.53	2.29
20 - 40	4.59	3.98	9.1	0.18	0.01	2.16	0.14	0.05	0.03	0.12	0.34	0.61	0.50	0.85	1.82

3.3.2. Soil chemical analysis

3.3.2.1. Soil pH

In the N × P experiment, N application significantly ($p<0.001$) decreased pH (H_2O) at 0 – 20 cm by 0.37 pH units, from 5.67 to 5.30, at the highest N application rate of 60 mg/kg (Figure 3.3a). A slighly larger significant ($p<0.001$) decrease in pH (H_2O) at 20 – 40 cm was observed. pH (H_2O) at this depth decreased from 5.64 to 5.19 at 60 mg/kg N. At an N application rate of 60 mg/kg, pH (KCl) at 0 – 20 cm decreased slightly but significantly ($p=0.0240$) by 0.12 pH units (Figure 3.3b). A highly significant ($p=0.001$) decrease of the same magnitude, was observed at 20 – 40 cm (0.14 pH unit decrease).

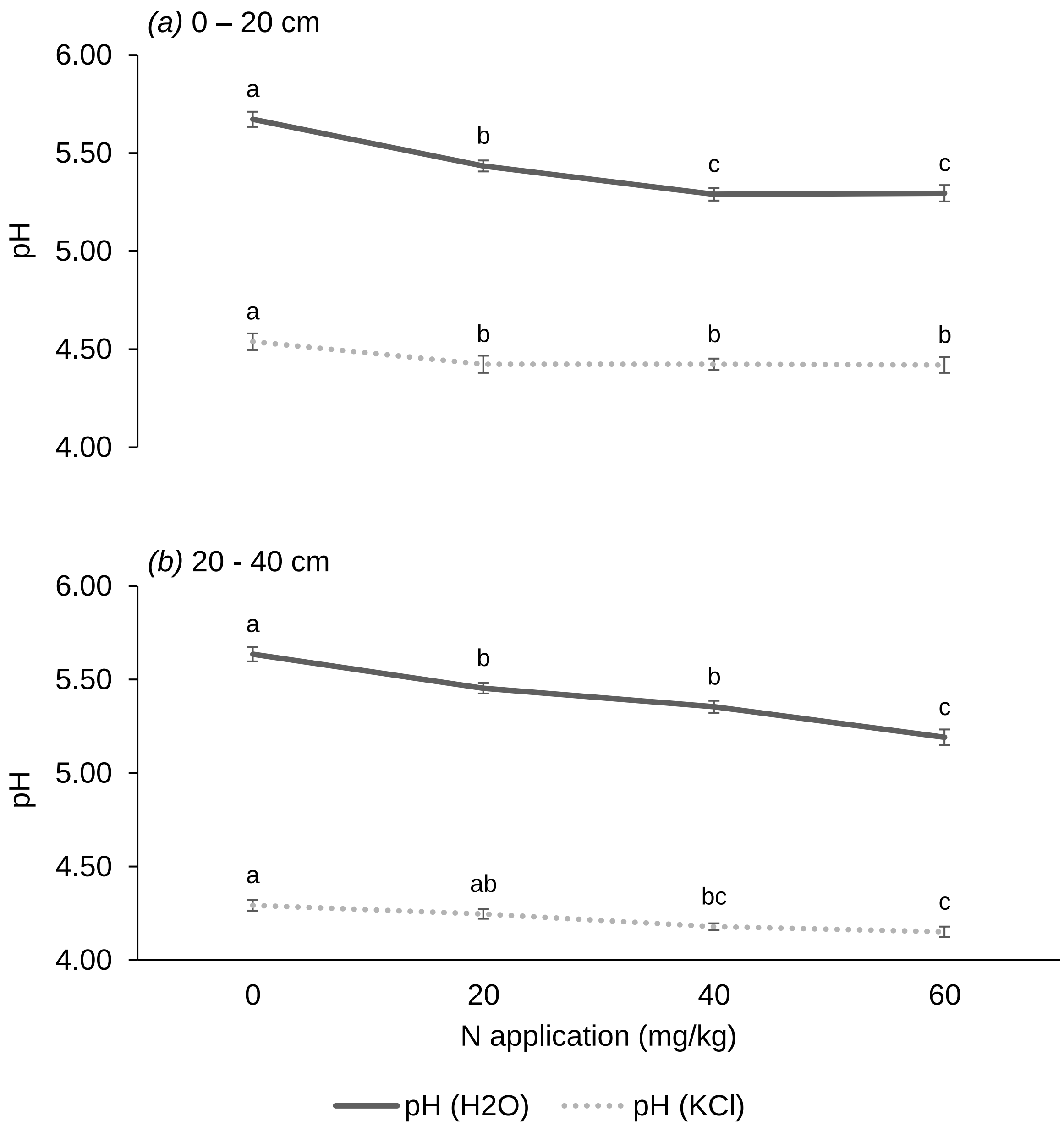

Figure 3.3. The effect of N application on pH (KCl) and pH (H_2O) at (a) 0 – 20 and (b) 20 – 40 cm. Letters denote significant differences between treatments within each data series.

Soil pH (H_2O) in the K × NP experiment significantly decreased at both depths with the application of 20 mg/kg N and 30 mg/kg P; at 0 – 20 cm from 5.67 to 5.48 (p=0.002) (Figure 3.4a) and at 20 – 40 cm from 5.58 to 5.40 (p=0.003) (Figure 3.4b). A slight, but statistically insignificant decrease in pH (KCl) at both depths (Figure 3.4ab).

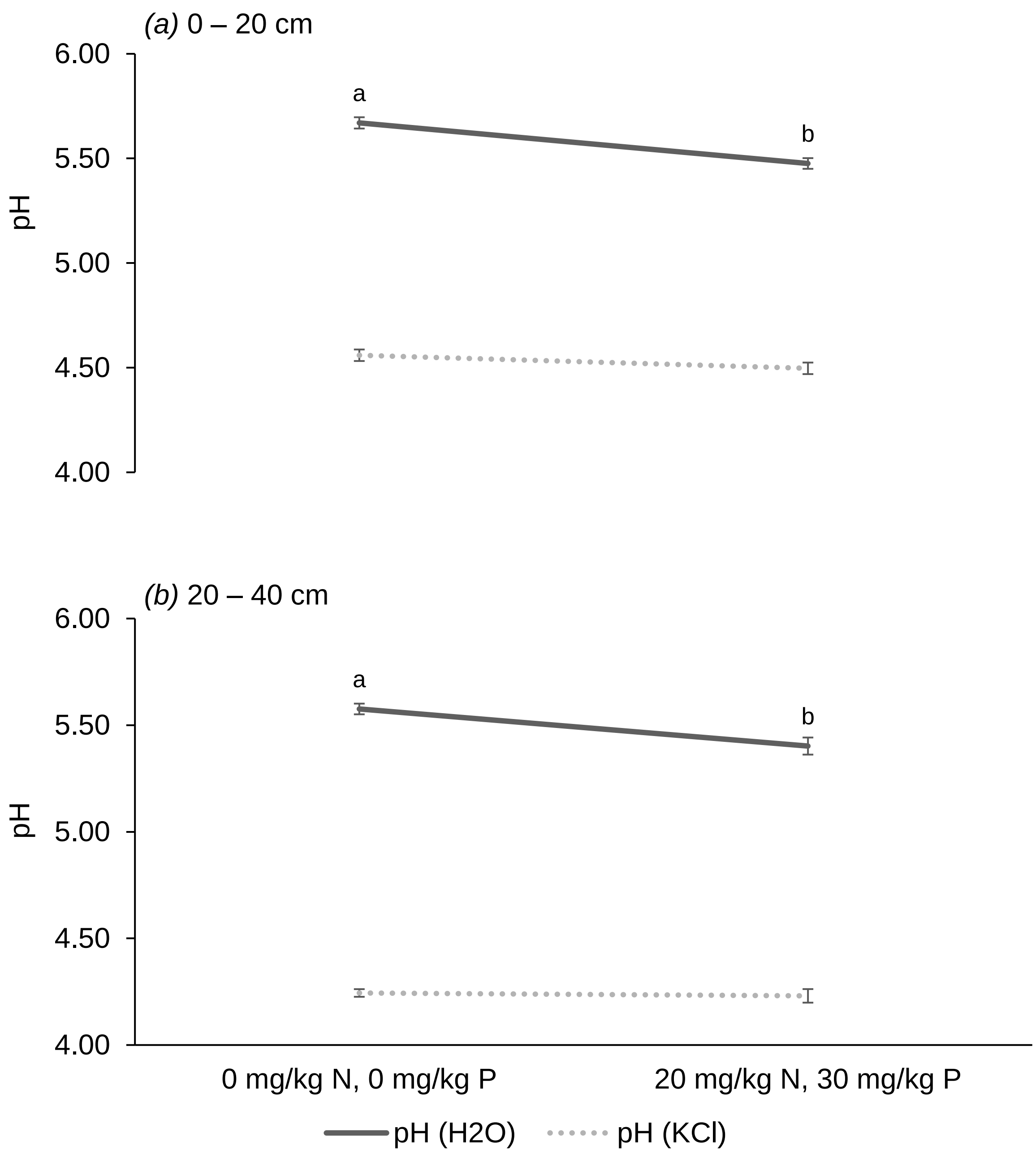

Figure 3.4. pH (KCl) and pH (H_2O) at (a) 0 – 20 and (b) 20 – 40 cm. Different letters indicate significant differences between treatments within each data series.

Although the initial hydrolysis of urea increases the alkalinity of the soil, the subsquent process of nitrification of ammonium to nitrate releases H^+ and the overall process is acid-forming:

$$CO(NH_2)_2\,(urea) + 4O_2 \rightarrow 2H^+ + 2NO_3^- + H_2O$$

The uptake of ammonia by plants and the leaching of nitrate accompanied by basic cations may also be responsible for this decline in pH. Higher pH values at 0 – 20 cm compared to those at 20 – 40 cm can be attributed to basic cation cycling due to deposition of organic materials by natural vegetation prior to the field being used for cultivation (Smith, 2014). Even at the highest N applications, pH (H_2O) fell well within the range of pH 4.5 – 5.5 considered ideal for rooibos growth (Joubert et al., 1987). The continued application of N in the form of urea may have implications for the availibility of basic cations due to leaching and a decrease in CEC, as well as the availablility of micronutrients and P (Smith et al., 2017). However, the rooibos plant is quite tolerant of acidic soil. According to Muofhe & Dakora (1999a), the rhizobia with which the rooibos plant forms a symbiosis is tolerant of extremely low pH values. Rooibos also possesses the ability to raise the pH in its rhizosphere, which assists in the colonisation of rooibos roots by these rhizobia.

3.3.2.2. Electrical conductivity

N application was associated with a significant ($p<0.001$) increase in EC at both 0 – 20 and 20 – 40 cm (Figure 3.5). An increase of 0.01 dS/m was observed at 0 – 20 cm and 0.03 dS/m at 20 – 40 cm. This can be attributed to the increase in nitrate and ammonia. In the K × NP experiment, K application had a significant effect ($p<0.001$) only at 20 – 40 cm, increasing EC by 0.02 dS/m at an application rate of 40 mg/kg (Figure 3.6). EC values remained well below the threshold over which a soil is considered saline (4 dS/m) in spite of this increase.

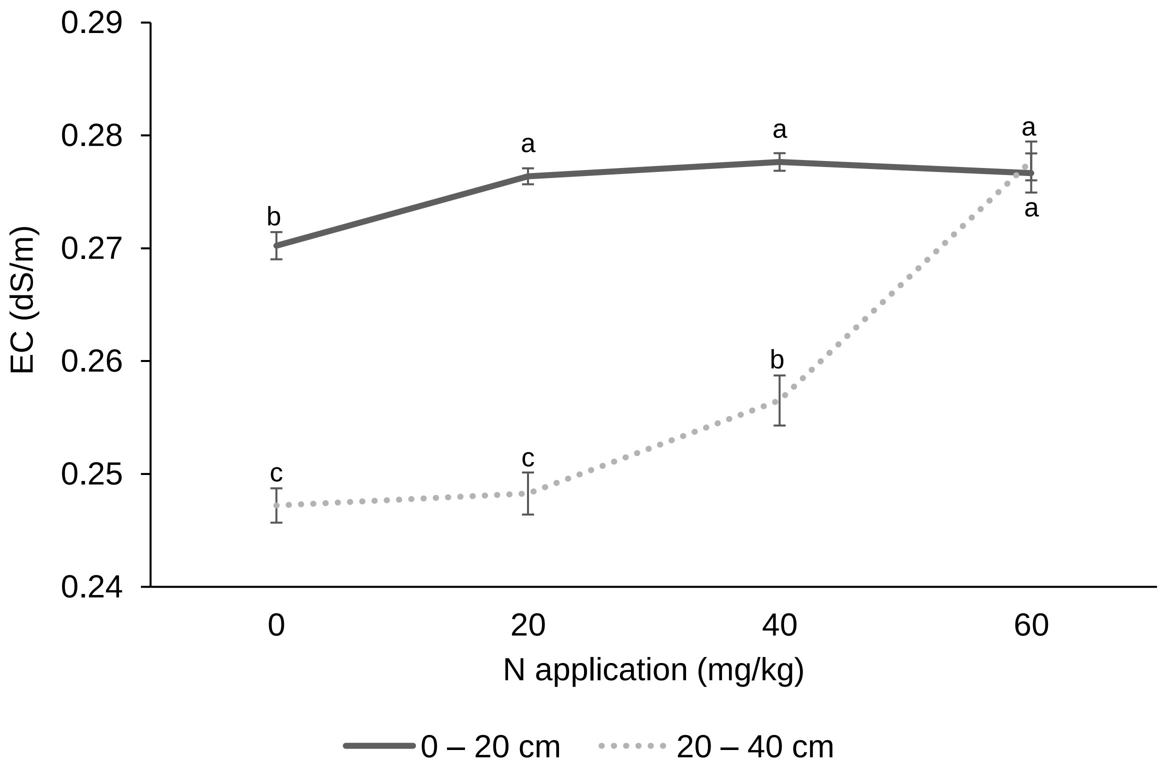

Figure 3.5. The effect of N application on EC at 0 – 20 cm in the N × P experiment. Different letters indicate significant differences between treatments within each data series.

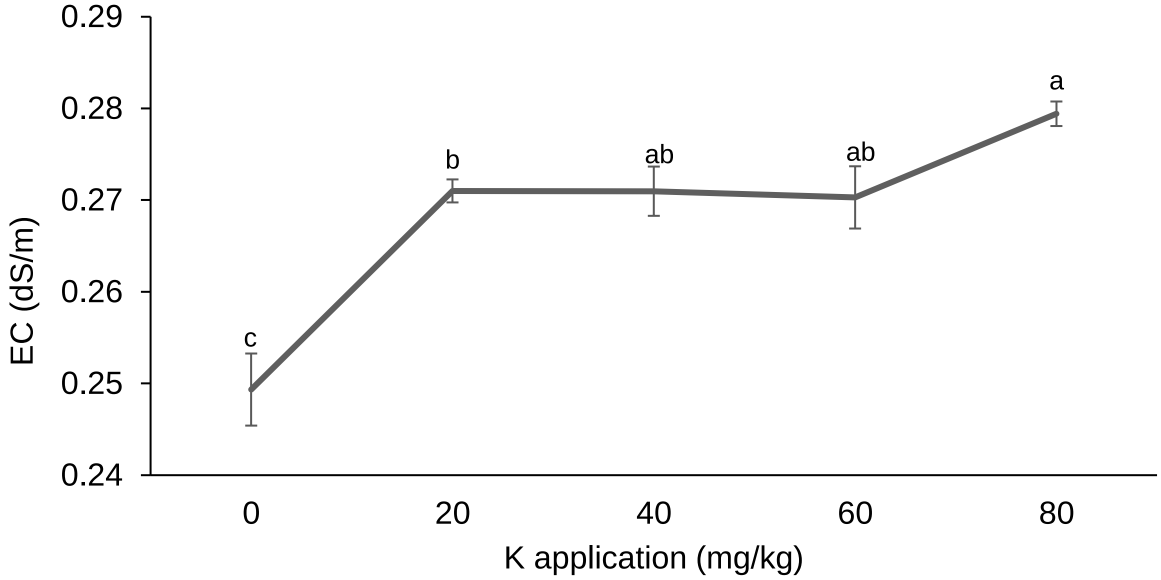

Figure 3.6. The effect of K application on EC at 20 – 40 cm in the K × NP experiment. Different letters indicate significant differences between treatments.

3.3.2.3. Total C

Neither N nor P application in the N × P experiment had significant effects on total soil carbon with P-values of 0.851 and 0.846 respectively. The application of K in the K × NP experiment at rates of 20 – 40 mg/kg had a small, but significant effect on total soil carbon, increasing it by 9.5% from 0.21% to 0.23% (Figure 3.7). This effect is most likely due to the increase in root biomass in these treatments, since the response curve closely resembles that of biomass response.

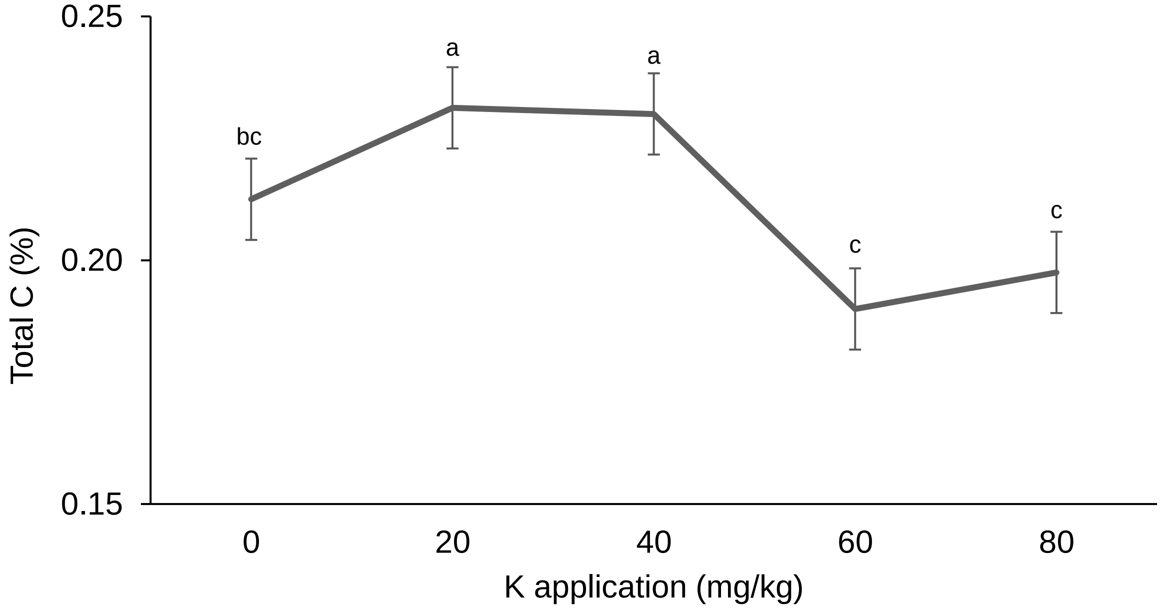

Figure 3.7. Effect of K application on total soil C in the K × NP experiment. Different letters indicate significant differences between treatments.

3.3.2.4. Exchangeable cations

P application had a significant effect ($p<0.001$) on exchangeable Ca at 0 – 20 cm, increasing it from 0.53 to 0.69 $cmol_c/kg$ with the addition of increasing amount of P fertiliser up to 60 mg/kg (Figure 3.8). The Ca content in TSP fertiliser can be identified as the source of this exchangeable Ca. An increase in the proportion of this element on exchange sites must be accompanied by a concomitant decrease in other cations, most likely H^+ and Al^{3+}.

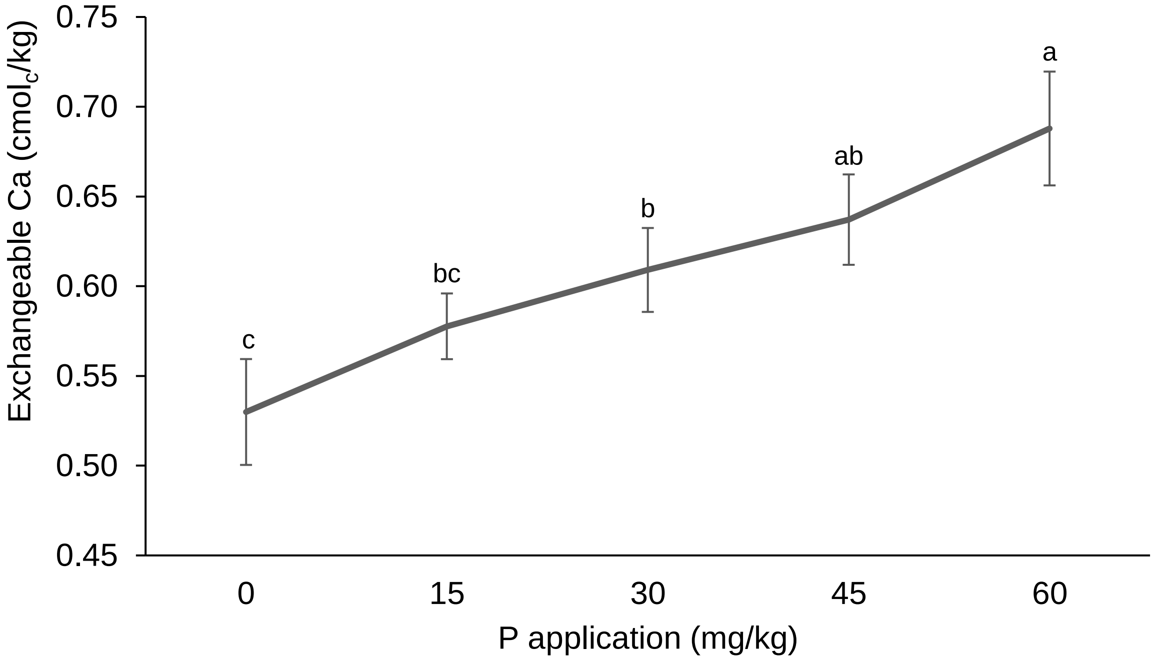

Figure 3.8. The effect of P application in the N × P experiment on exchangeable calcium at 0 – 20 cm. Different letters indicate significant differences between treatments.

In the K × NP experiment the addition of K had a significant effect ($p=0.006$) on exchangeable Ca, decreasing both with and without the addition of N and P. This can be ascribed to the applied K competing with Ca for exchange sites. The application of 20 mg/kg N and 30 mg/kg P significantly ($p=0.004$) increased exchangeable Ca at 0 – 20 cm from an average of 0.45 to 0.55 $cmol_c/kg$. This effect was observed at K application rates from 0 – 40 mg/kg, although higher rates of K application counteracted the increase due to N and P application, depressing it significantly (Figure 3.9).

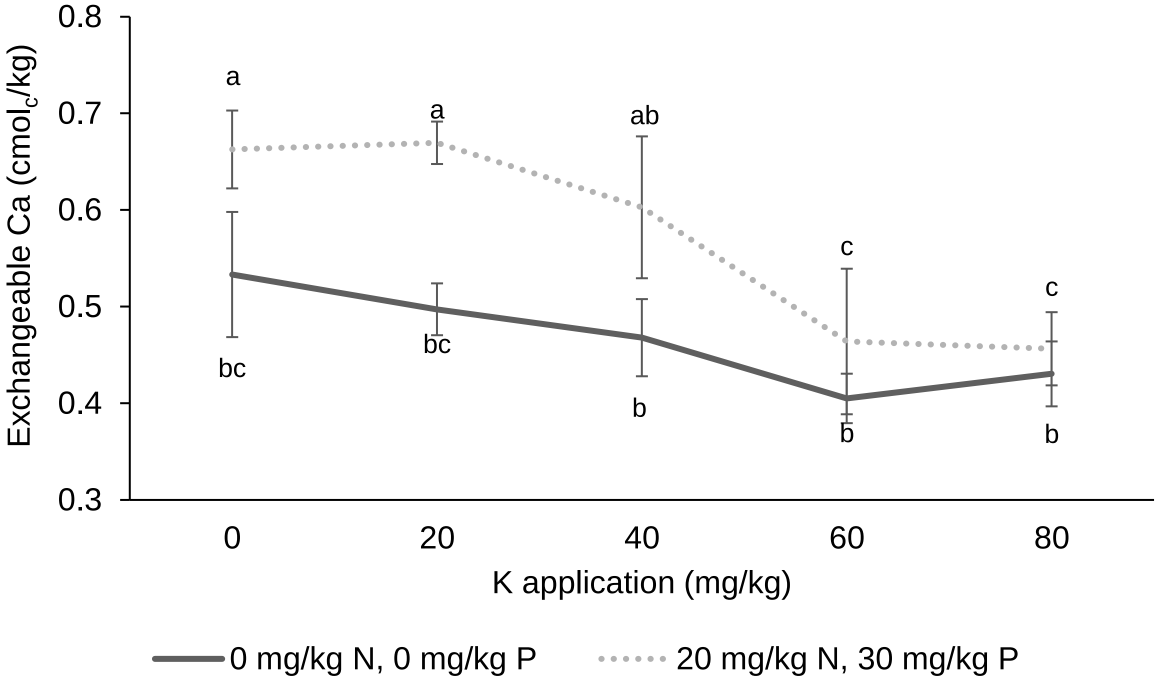

Figure 3.9. The effect of K application with and without NP application in the K × NP experiment on exchangeable Ca at 0 - 20 cm. Different letters indicate significant differences between treatments.

The increase in exchangeable Ca was accompanied by a concomitant decrease in exchangeable acidity (Figure 3.10). Values decreased significantly ($p<0.001$) with the addition of TSP fertiliser, which decreased exchangeable acidity by approximately 0.0083 cmol$_c$/kg for every 10 mg/kg P applied.

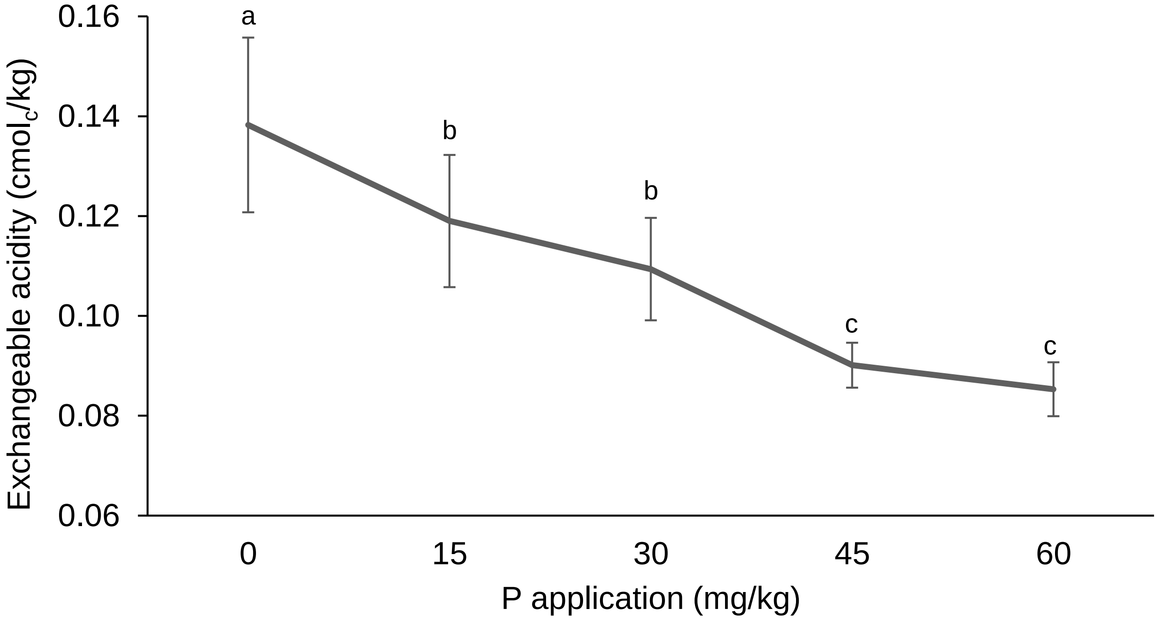

Figure 3.10. The effect of P application on exchangeable acidity at 0 - 20 cm in the N × P experiment. Different letters indicate significant differences between treatments.

K application had a significant effect on exchangeable acidity in the K × NP experiment. At 20 mg/kg K, H^+ and Al^{3+} on exchange sites was displaced by K^+, decreasing exchangeable acidity from 0.16 to 0.08 $cmol_c$/kg. Exchangeable acidity remained stable at 0.08 – 0.09 $cmol_c$/kg with increasing K application (Figure 3.11).

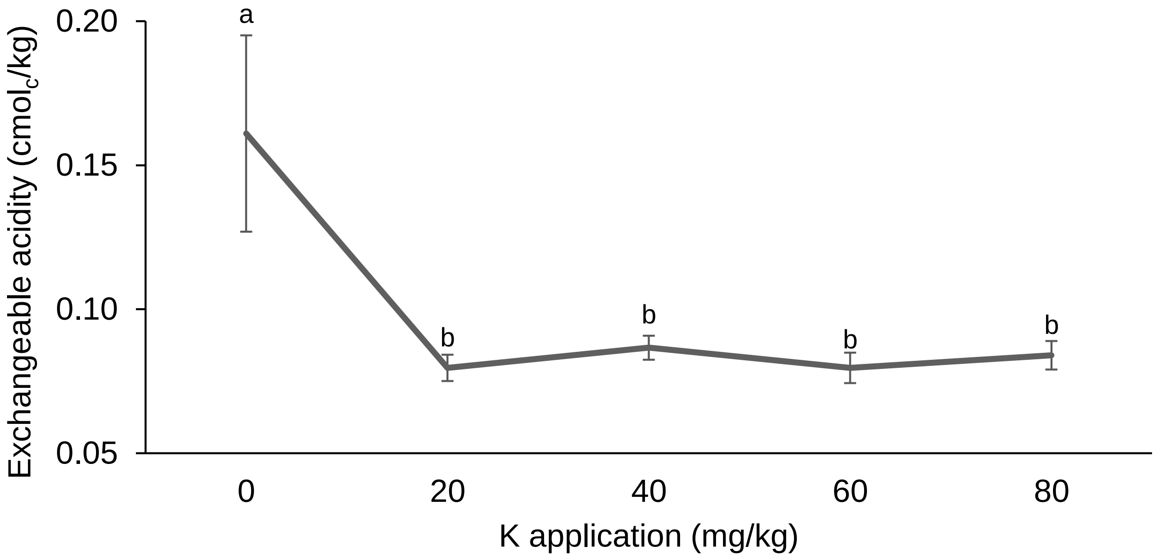

Figure 3.11. Effect of K application on exchangeable acidity at 0 – 20 cm in the K × NP experiment. Different letters indicate significant differences between treatments.

P application had a significant effect ($p<0.001$) on exchangeable Mg in the N × P experiment. Exchangeable Mg decreased from 0.21 – 0.22 to 0.17 $cmol_c$/kg at a P application rate higher than 45 mg/kg (Figure 3.12). This can be attributed to Ca in the TSP fertiliser replacing Mg at this higher concentration.

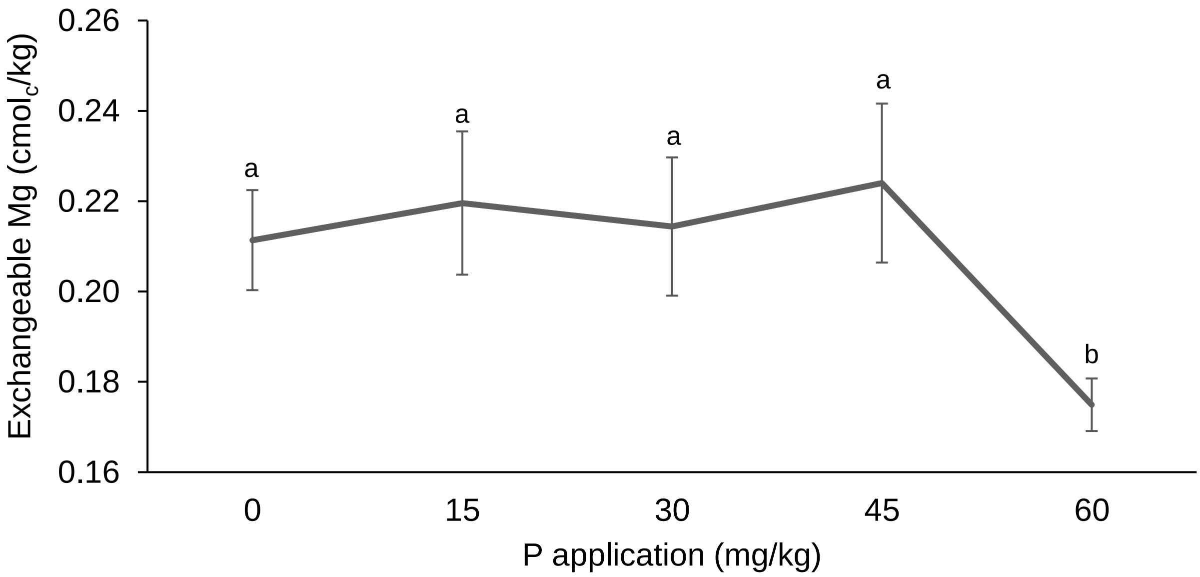

Figure 3.12. The effect of P application on exchangeable Mg at 0 - 20 cm in the N × P experiment. Different letters indicate significant differences between treatments

The application of K in the K × NP experiment was also associated with a significant decrease in exchangeable Mg ($p<0.001$). This can be ascribed to K replacing Mg at exchange sites (Figure 3.13). A decrease in soil Mg should not hinder rooibos plant growth, as the application of this nutrient has been shown in a previous study to supress the growth of rooibos (Joubert et al., 1987).

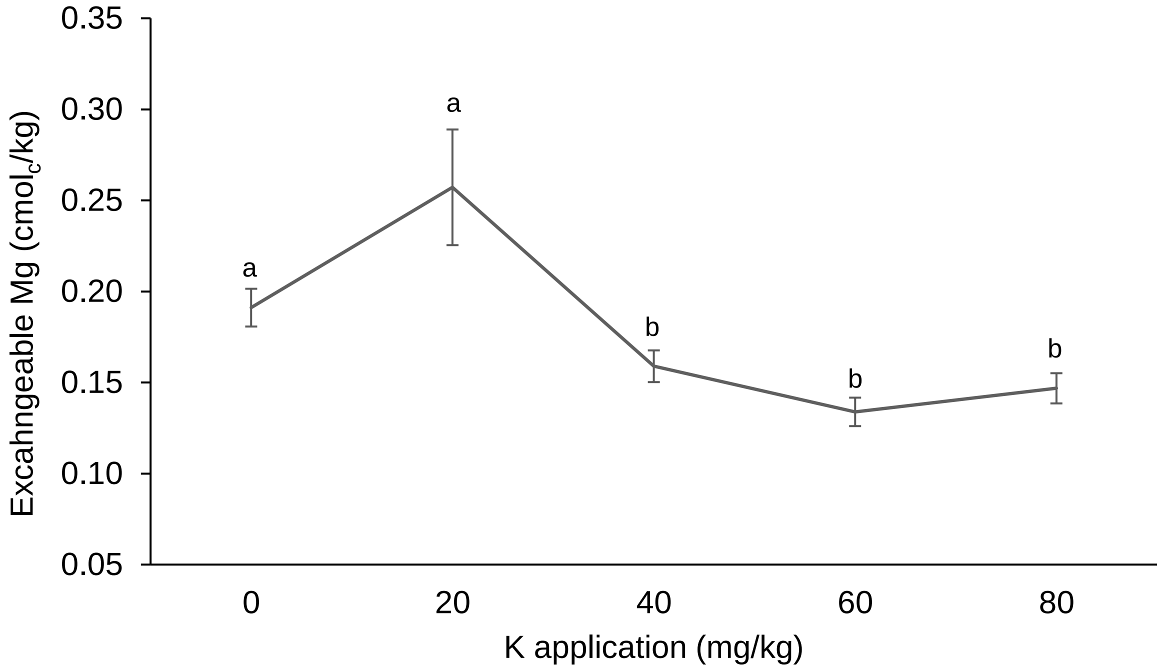

Figure 3.13. The effect of K application on exchangeable Mg at 0 - 20 cm in the K × NP experiment. Different letters indicate significant differences between treatments.

The application of N and P in the N × P experiment had no effect on exchangeable K, where values remained at 0.057 – 0.100 $cmol_c$/kg. On the contrary, in the K × NP experiment, K application had a highly significant effect ($p<0.001$) on exchangeable K, which increased from 0.09 $cmol_c$/kg in the control treatment to 0.23 $cmol_c$kg at the highest application rate – an increase of 0.018 $cmol_c$/lg K for every 10 mg/kg applied (Figure 3.14).The fertiliser treatments had no significant effect on exchangeable Na (data not shown). Values ranged from 0.061 – 0.16 $cmol_c$/kg in the N × P experiment and 0.058 – 0.078 $cmol_c$/kg in the K × NP experiment. ECEC values ranged from 0.95 – 1.33 $cmol_c$/kg, in the N × P experiment and 0.87 – 1.27 $cmol_c$/kg in the K × NP experiment.

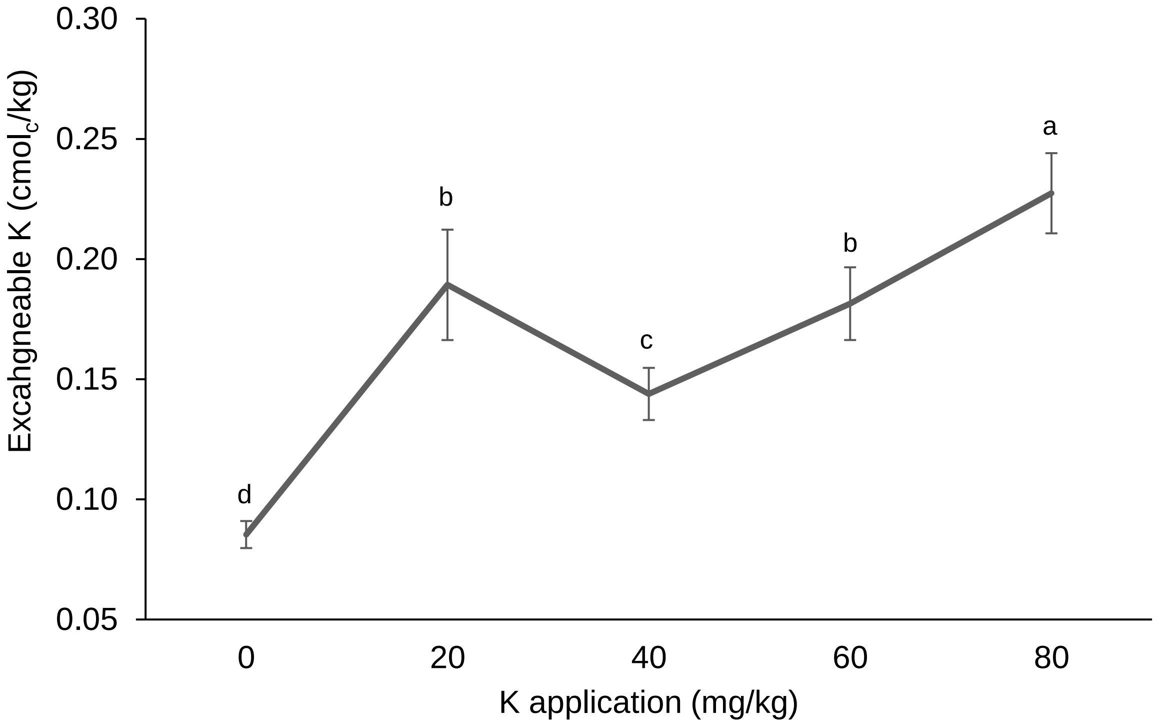

Figure 3.14. The effect of K application on exchangeable K at 0 - 20 cm in the K × NP experiment. Different letters indicate significant differences between treatments.

3.3.2.5. Plant-available P

As can be expected, the application of P fertiliser significantly increased plant-available (Bray II) P at 0 – 20 ($p<0.001$) and 20 – 40 cm ($p<0.001$). Bray II P at 0 – 20 cm increased by 0.36 mg/kg (R^2 = 0.8155) for every mg/kg P added, while at 20 – 40 cm, every mg P added resulted in an increase in plant available P of 0.72 mg/kg (R^2 = 0.9066) (Figure 3.15). The higher P levels observed at 20 – 40 cm can be accounted for by the manner in which the P fertiliser was applied, i.e. ploughing into 20 cm depth or deeper. When P levels at the total depth of 0 – 40 cm are examined, P is found to increase by 0.98 mg/kg for every mg P added. The P levels intended for this study were thus attained, and it would appear that little uptake by the rooibos plants or P-fixation as Al or Fe-compounds has occurred.

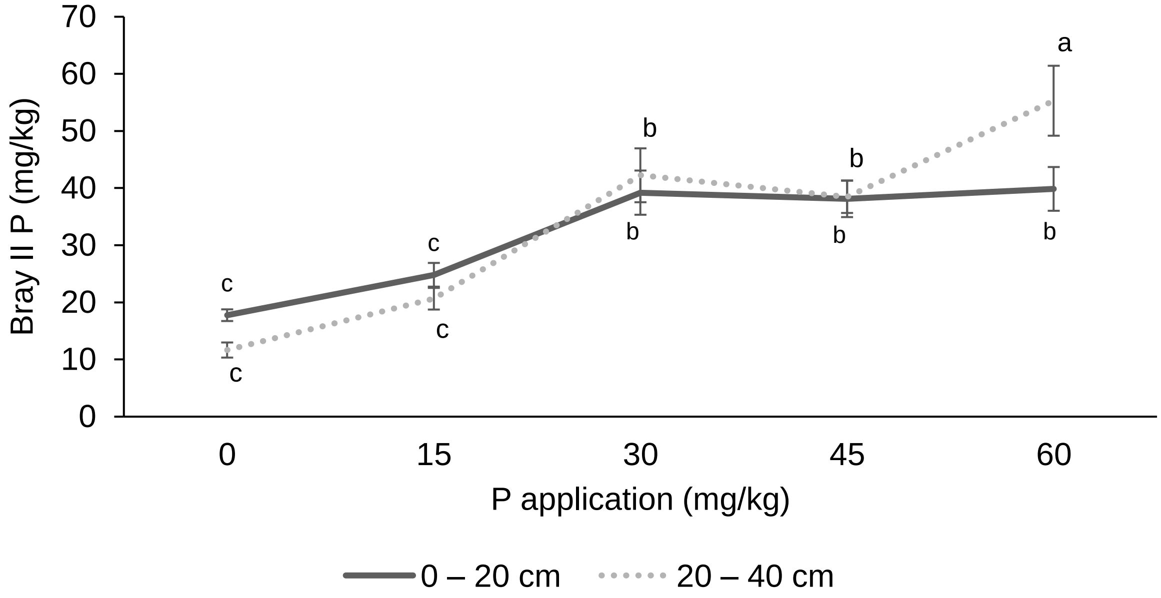

Figure 3.15. The effect of P application on plant-available P (Bray II) at 0 – 20 cm and 20 – 40 cm in the N × P experiment. Different letters indicate significant differences between treatments within each data series.

Similarly, in the K × NP experiment, significant (p<0.001) increases in P level were observed at both 0 – 20 cm and 20 – 40 cm, with the application of 20 mg/kg N and 30 mg/kg P. Plant available P increased by 0.62 and 0.49 mg/kg at 0 – 20 and 20 – 40 cm respectively, for each mg/kg P applied (Figure 3.16). P over the total depth of 0 – 40 cm increased by approximately 1 mg/kg for each mg/kg applied. It must be noted that the Bray II P level in the control treatment of this experiment are approximately 10 mg/kg higher than those observed in the preliminary soil chemical analysis. Although this is not likely to be a methodological error, this discrepancy is being investigated and soil samples have been sent for analysis by a third party.

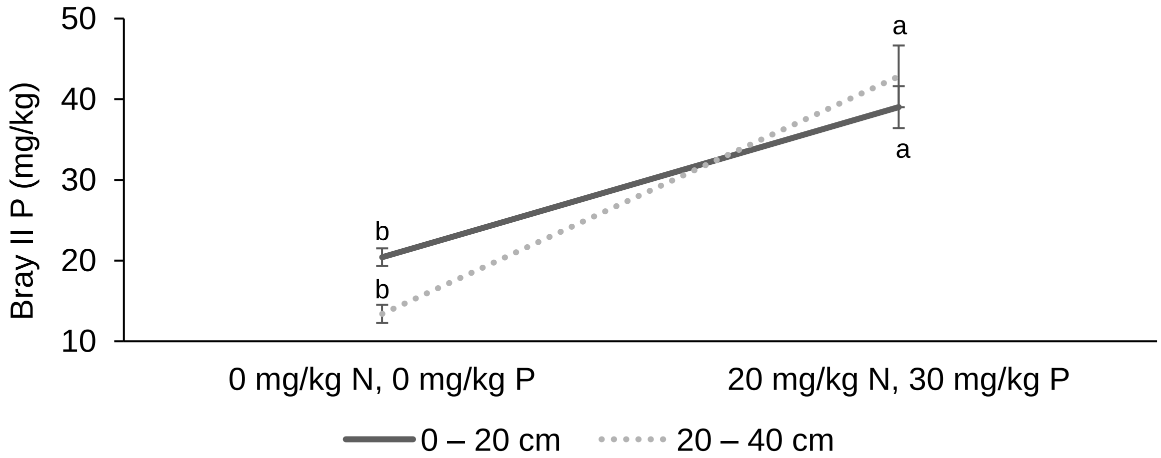

Figure 3.16. The effect of N and P application on plant-available (Bray II) P in the K × NP experiment. Different letters indicate significant differences between treatments within each data series.

3.3.2.6. Plant-available micronutrients

The addition of N in the N × P experiment had a significant effect (p=0.018) on plant-available Cu, increasing it from 0.35 to 0.42 – 0.45 mg/kg (Figure 3.17). This effect is likely due to the effect N application had on pH lowering it with increased application (Figure 3.3) which may increase the solubility of Cu via reduction. These soil Cu levels are similar to those found in other areas where rooibos is cultivated (Smith, 2014; Muofhe & Dakora, 1999). None of the treatments used in the experiments had a significant effect on plant-available manganese, which ranged from 8.14 – 16.1 mg/kg (N × P) and 5.29 – 10.8 (K × NP). These values correspond well to the topsoil Mn values in a previous study at Nardouwsberg (Smith, 2014).

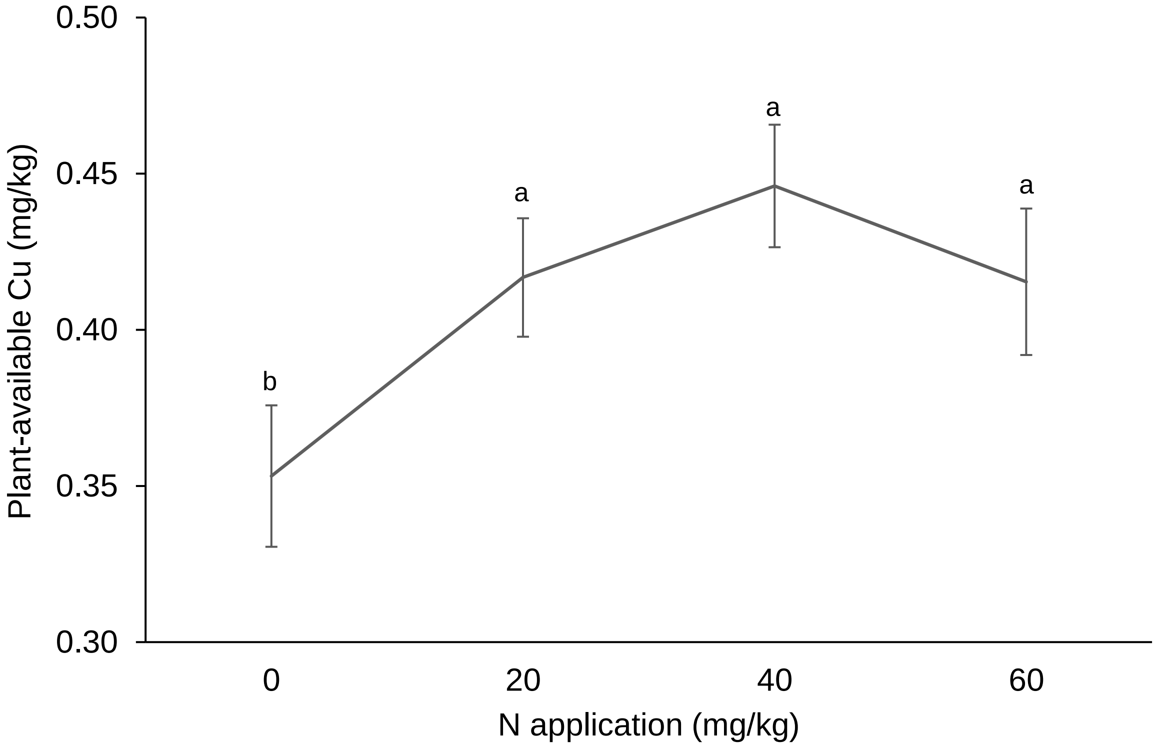

Figure 3.17. The effect of N application on plant-available Cu at 0 - 20 cm in the N × P experiment. Different letters indicate significant differences between treatments.

Plant-available Zn was significantly affected by the level of P application in the N × P experiment. Zn levels increased from 0.16 mg/kg in the control to 0.26 mg/kg at 60 mg/kg P (Figure 3.18). Furthermore, the application of 20 mg/kg N and 30 mg/kg P significantly (p=0.014) increased levels in the K × NP experiment from 0.188 to 0.252 mg/kg, an increase similar to that observed at 30 mg/kg P in the N × P experiment. This relationship is likely due to trace amounts of Zn in the TSP fertiliser used for this trial. Zn levels in the control treatment were somewhat lower than those reported by Smith (2016).

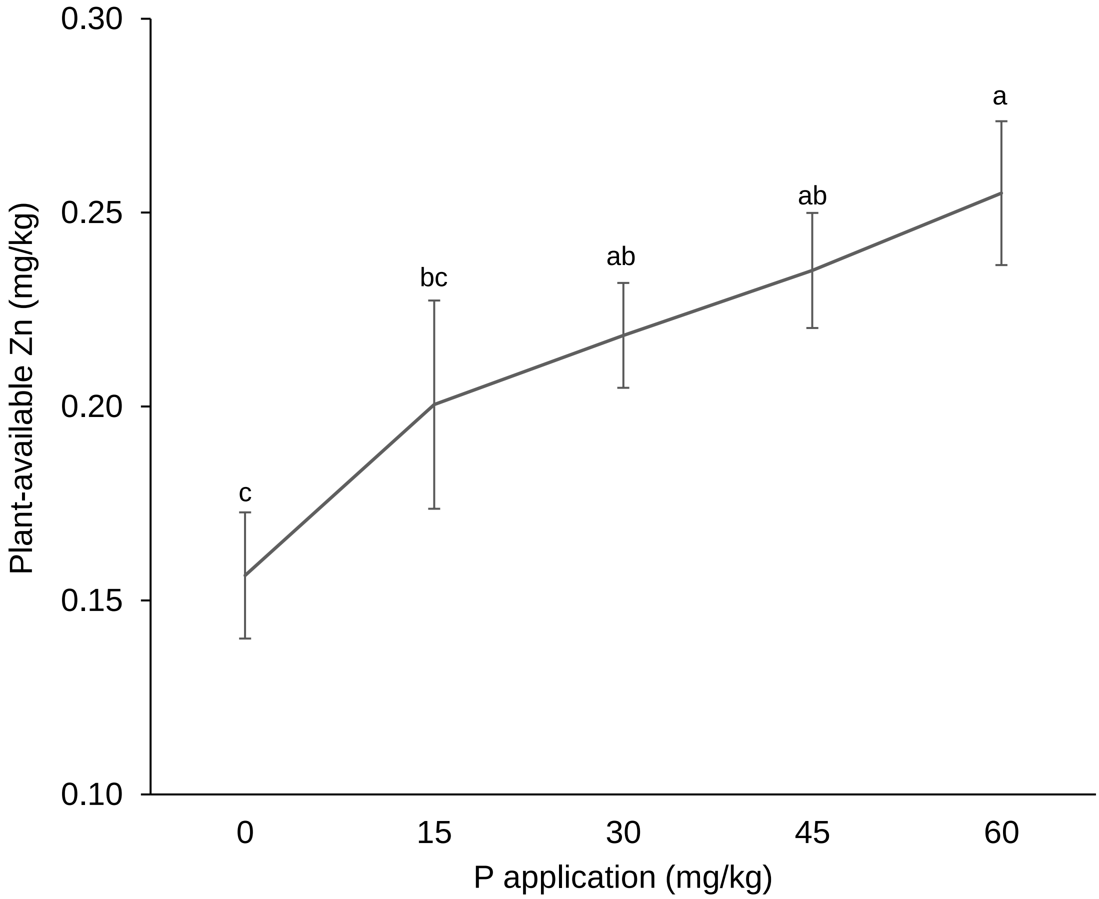

Figure 3.18. The effect of P application on plant-available Zn at 0 - 20 cm in the N × P experiment. Different letters indicate significant differences between treatments.

3.3.3. Foliar analysis

3.3.3.1. Foliar N

The application of N had no statistically significant effect on foliar N concentration in the N × P experiment. P application had a statistically significant effect (p=0.009) on the accumulation of foliar N. Foliar N levels increased from 2.26 % in the control to 2.34 – 2.49% at application rates of 30 mg/kg P and higher (Figure 3.19) indicating that P supply may influence the uptake of N. In general, foliar N values in this study were nearly twice as high as those reported for mature cultivated rooibos plants in both their winter and summer growth phases in the Wuppertal region (Lötter et al., 2014) and those reported for 1 – 2-year-old rooibos plants in the Clanwilliam area (Smith, 2014) even in the control treatment. This may be due to the preparation method for foliar analysis used in this study, where leaves were stripped from the branches to avoid a tissue dilution effect. Foliar N was also positively correlated with the supply of plant-available P at 20 – 40 cm (R^2 = 0.5330) (Figure 3.20) but only weakly positively correlated with foliar P (R^2=0.3545).

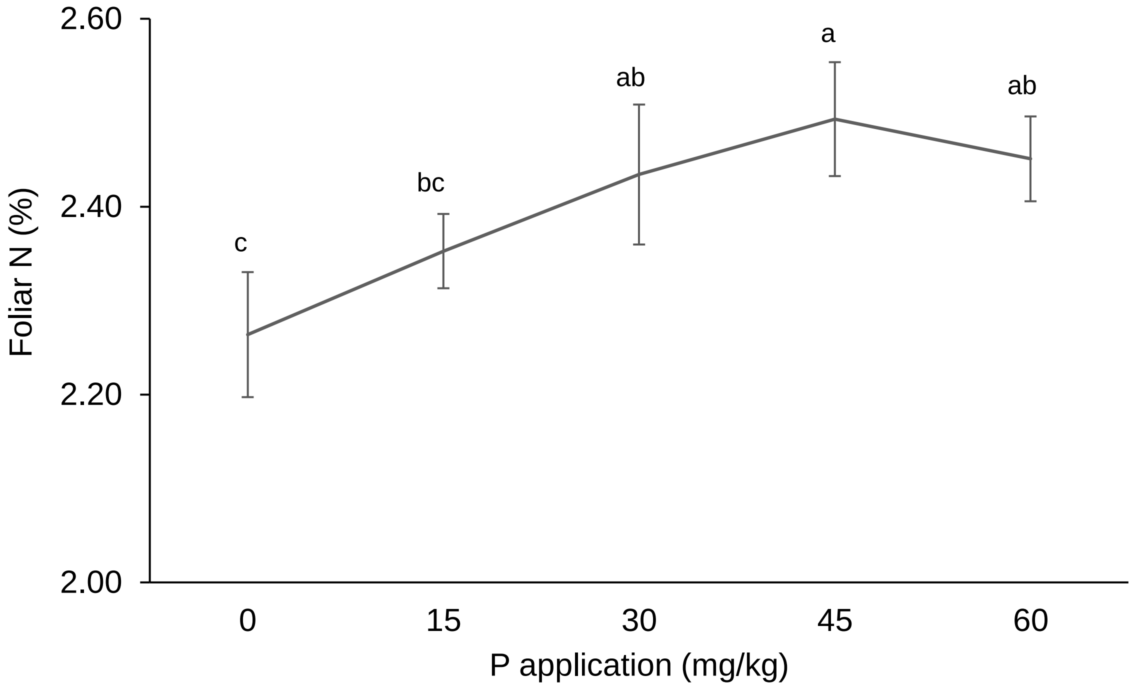

Figure 3.19. The effect of P application on foliar N in the N × P experiment. Different letters indicate significant differences between treatments.

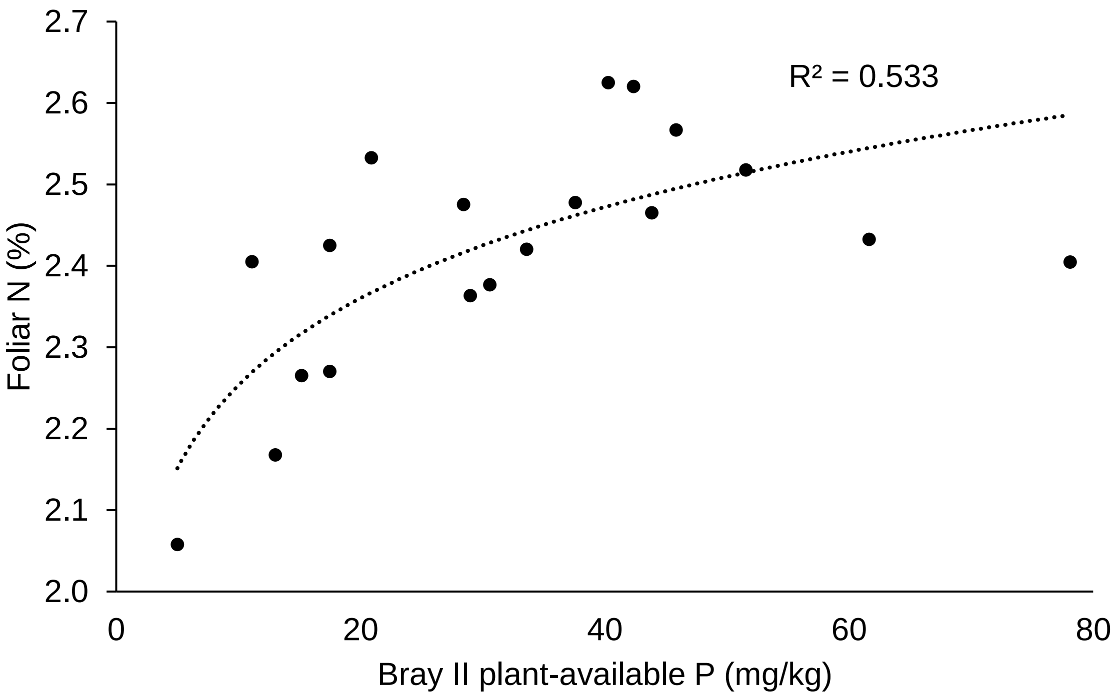

Figure 3.20. Correlation between foliar N and Bray II P at 0 - 20 cm in the N × P experiment.

No statistically significant effects on foliar N concentration were found in the K × NP experiment. Values ranged from 2.27 to 2.65 % with a mean of 2.44 ± 0.145 %.

3.3.3.2. Foliar P

The application of N did not have a significant effect on P uptake (p=0.178), contrary to the findings of Maistry et al. (2015). Furthermore, Foliar N and P were only weakly correlated (R^2 = 0.3545) in the N × P experiment in this study. In the K × NP study, the application of K had no effect on foliar P. However, the application of 20 mg/kg N and 30 mg/kg P significantly (p>0.001) increased foliar P from 0.060 to 0.086 %. P application had a highly significant effect on the foliar P content (p<0.001) in the N × P experiment. The addition of P at levels above 15 mg/kg significantly increased foliar P (Figure 3.21). At a P application rate of 60 mg/kg, foliar P content increased from a value of 0.07% in the control to 0.11%. Foliar P was also linearly correlated (R^2 = 0.5270) with plant-available P at 20 – 40 cm, confirming that rooibos is unable to regulate P uptake when this nutrient is supplied in abundance (Hawkins et al., 2008).

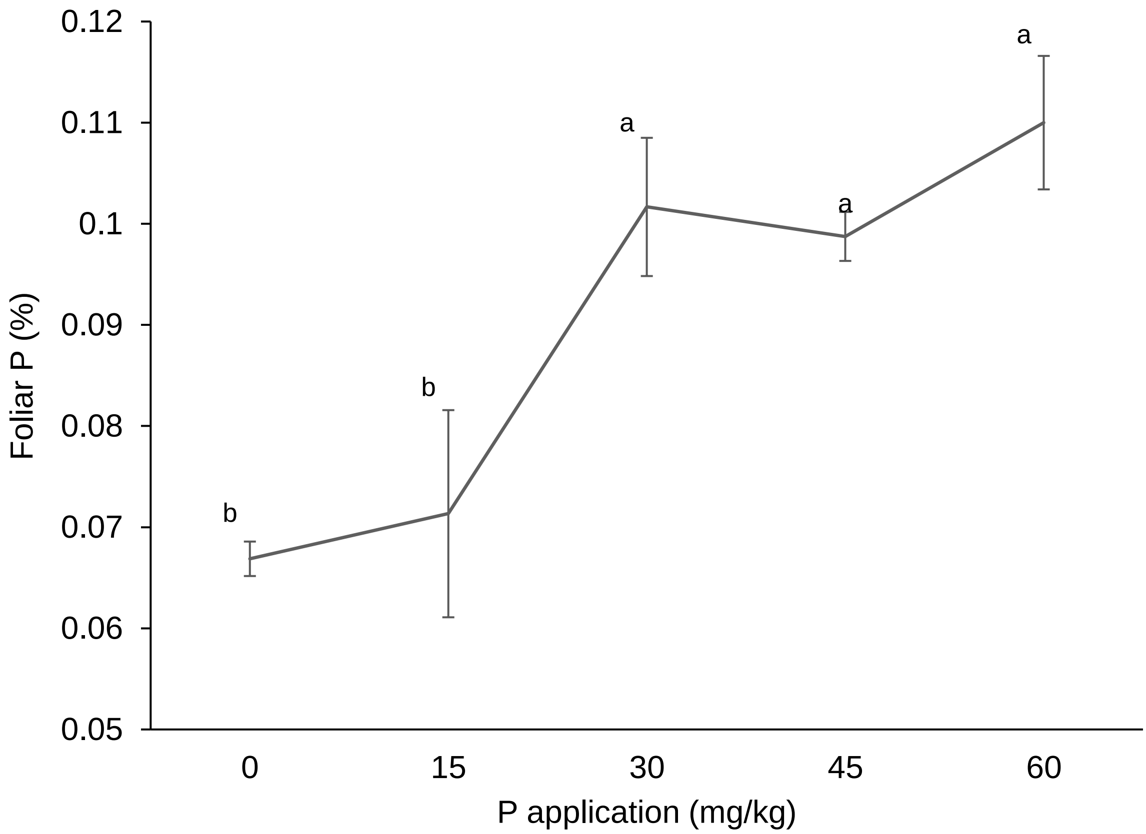

Figure 3.21. The effect of P application on foliar P content in the N × P experiment. Different letters indicate significant differences between treatments.

3.3.3.3. Foliar K

The application of N in the N × P experiment had a highly significant effect in foliar K concentration ($p<0.001$), although no clear trend can be discerned. The highest K levels occurred in plants fertilised with 40 mg/kg K (0.52%) (Figure 3.22). In the K × NP experiment, the application of 20 mg/kg N and 30 mg/kg P at K applications between 0 – 80 mg/kg decreased foliar K significantly ($p=0.006$) from 0.59 to 0.55 %. Interestingly, no relationship between K application and foliar K was found, this may be due a dilution effect, as plants fertilised with K were larger.

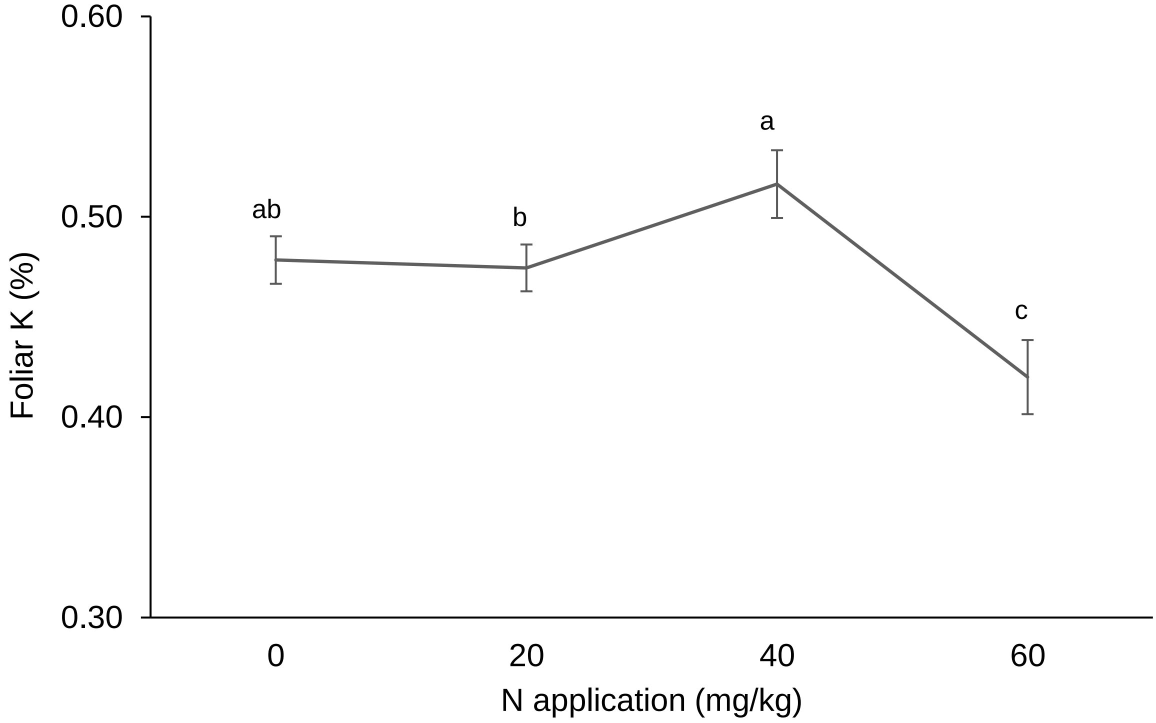

Figure 3.22. The effect of N application on foliar K in the N × P experiment. Different letters indicate significant differences between treatments.

3.3.3.4. Foliar Ca

No response to the application of NPK on foliar Ca was observed in this study. Concentrations ranged from 0.43 – 0.99 % with a mean of 0.63 ± 0.11 % in the N × P experiment and 0.42 – 0.68 % with a mean of 0.58 ± 0.10 % in the K × NP experiment. No correlations with other plant or soil parameters where found.

3.3.3.5. Foliar Mg

The application of N had no significant effect on foliar Mg content in the N × P experiment. P application in this experiment, however, had a significant effect on foliar Mg content ($p=0.002$). A significant increase, from 0.32% to 0.40 – 0.45% occurred with a P application rate of 15 mg/kg (Figure 3.23). No significant changes in foliar Mg occurred with increased P application above this level. The application of N and P in K × NP experiment increased foliar Mg from 0.32 to 0.36 %, although the increase was not statistically significant.

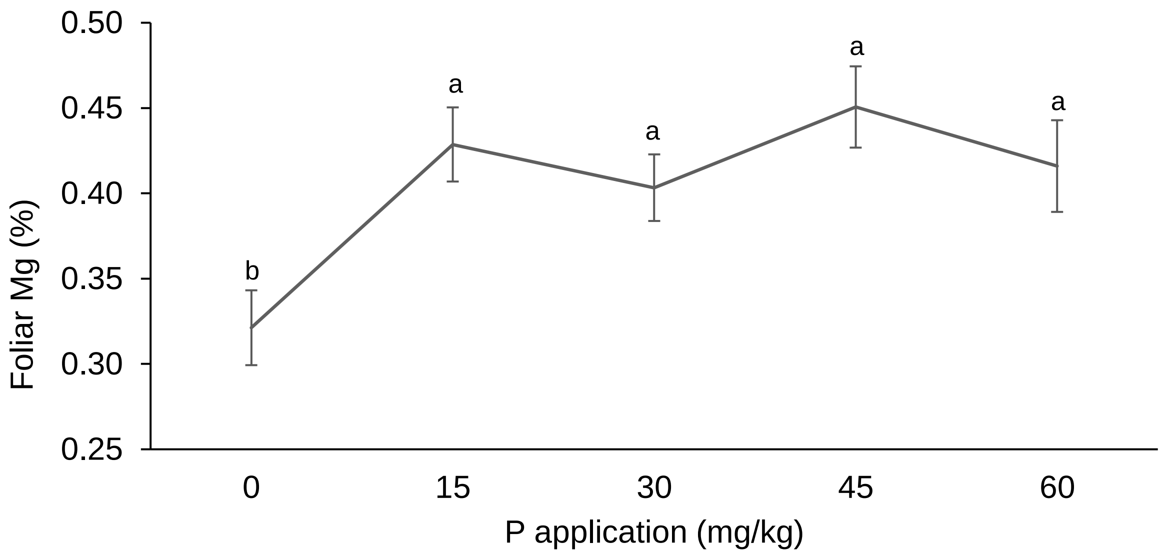

Figure 3.23. The effect of P application on foliar Mg content in the N × P experiment. Different letters indicate significant differences between treatments.

3.3.3.6. Foliar Na

None of the treatments in this study had a significant effect on foliar Na concentration (data not shown). Levels ranged between 3434 – 4640 mg/kg with a mean of 3997 ± 313.5 mg/kg in the N × P experiment. In the K × NP experiment, foliar Na ranged between 3545 – 4748 mg/kg with a mean concentration of 4141 ± 412 mg/kg. No correlation between soil Na and foliar Na was found. These values are higher than those found by Smith (2014) in one-year old plants in the same area.

3.3.3.7. Foliar Fe

The application of NPK had no significant effects on foliar Fe concentration in this study. Concentrations in the N × P experiment were between 178.7 and 269.5 mg/kg (mean = 223.9 ± 27.84 mg/kg), and 199.83 and 300.43 mg/kg (mean = 243.8 ± 38.3 mg/kg). A negative correlation between foliar Fe and foliar Mg was observed in this study, indicating a possible antagonism (Figure 3.24). No correlations between foliar Fe and other foliar and soil parameters were found.

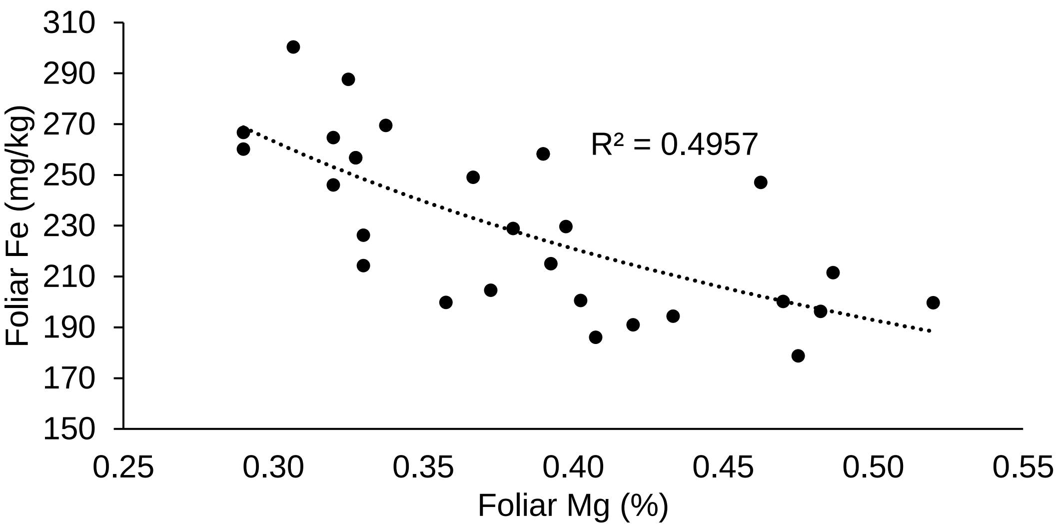

Figure 3.24. Correlation between foliar Fe and foliar Mg for all treatments in the N × P and K × NP experiments.

3.3.3.8. Foliar Cu

Both the application of N ($p<0.001$) and P ($p<0.001$) in the N × P experiment had highly significant effects on foliar Cu concentration. No interactive effects were observed. N application significantly increased foliar Cu from 1.25 mg/kg in the control to a maximum of 1.77 mg/kg at 40 mg/kg applied N, after which the concentration declined significantly 1.49 mg/kg at 60 mg/kg N (Figure 3.25). This increase in uptake may be due to the higher availability of Cu caused by the lowering of pH associated with the application of N fertiliser in this experiment (Figure 3.3).

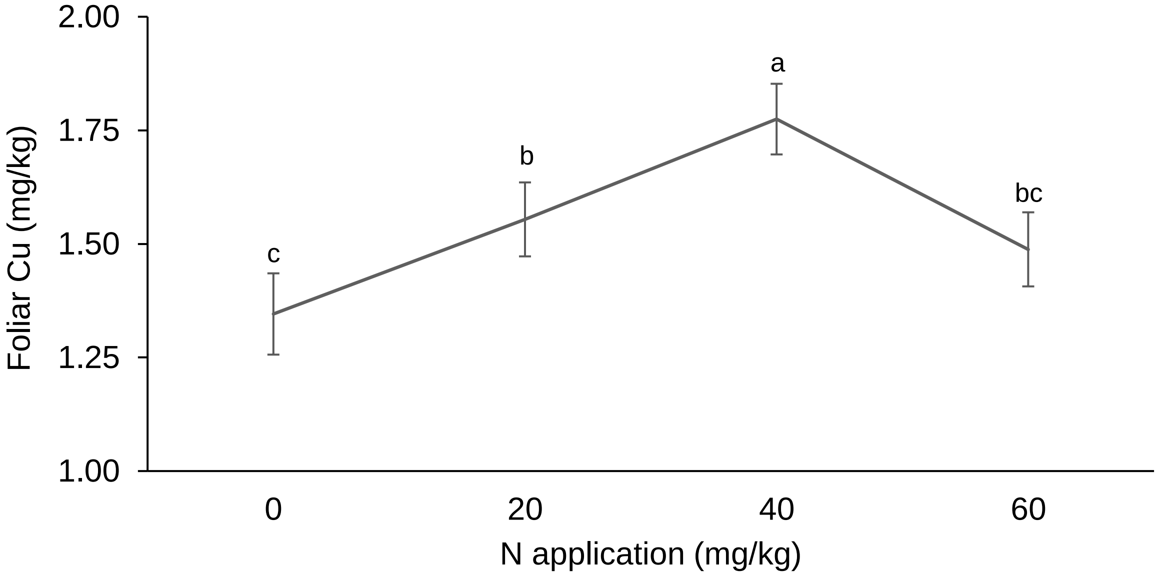

Figure 3.25. The effect of N application on foliar Cu content in the N × P experiment. Different letters indicate significant differences between treatments.

The application of P at all levels decreased foliar Cu concentration from 1.93 mg/kg 1.38 – 1.56 mg/kg in the N × P experiment (Figure 3.26). P applications of 15 mg/kg or higher decreased foliar Cu from 1.93 mg/kg to 1.41–1.56 mg/kg in N × P experiment. In the K × NP experiment K application of 20 mg/kg N and 30 mg/kg P over the range of 0 – 80 mg/kg K significantly ($p=0.006$) reduced mean foliar Cu from 2.01 to 1.56 mg/kg. A significant negative correlation ($R^2=0.5026$) was found between foliar Cu and foliar P (Figure 3.27) in the N × P and K × NP experiments, illustrating a clear Cu – P antagonism. High soil P levels are known to suppress the uptake of Cu via mycorrhiza (Kabata-Pendias, 2011), although in this experiment, foliar Cu was weakly correlated to soil P at 20 – 40 cm ($R = 0.2749$).

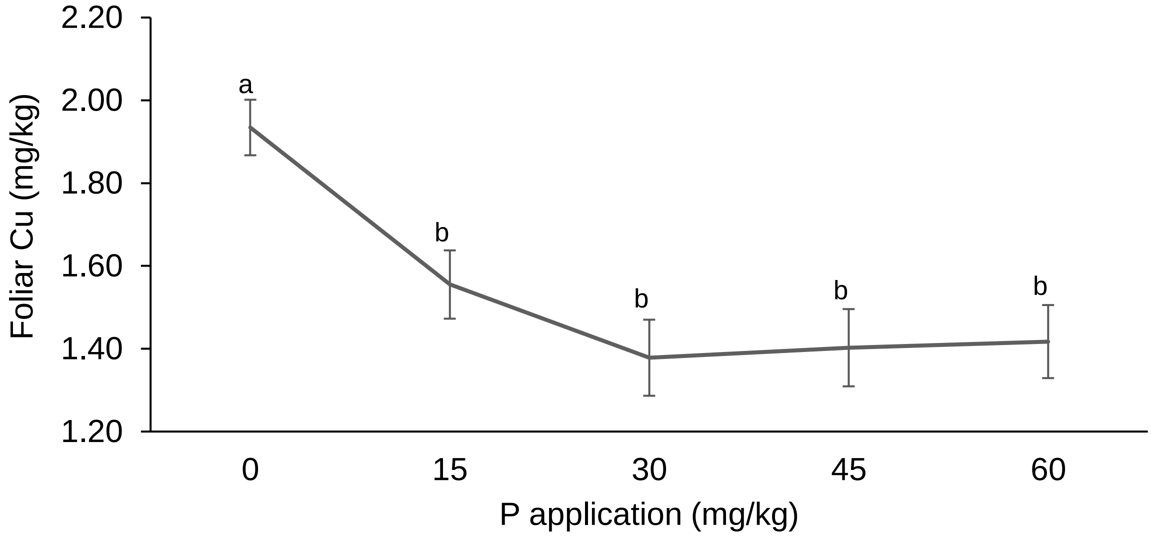

Figure 3.26. The effect of P application on foliar Cu content in the N × P experiment. Different letters indicate significant differences between treatments.

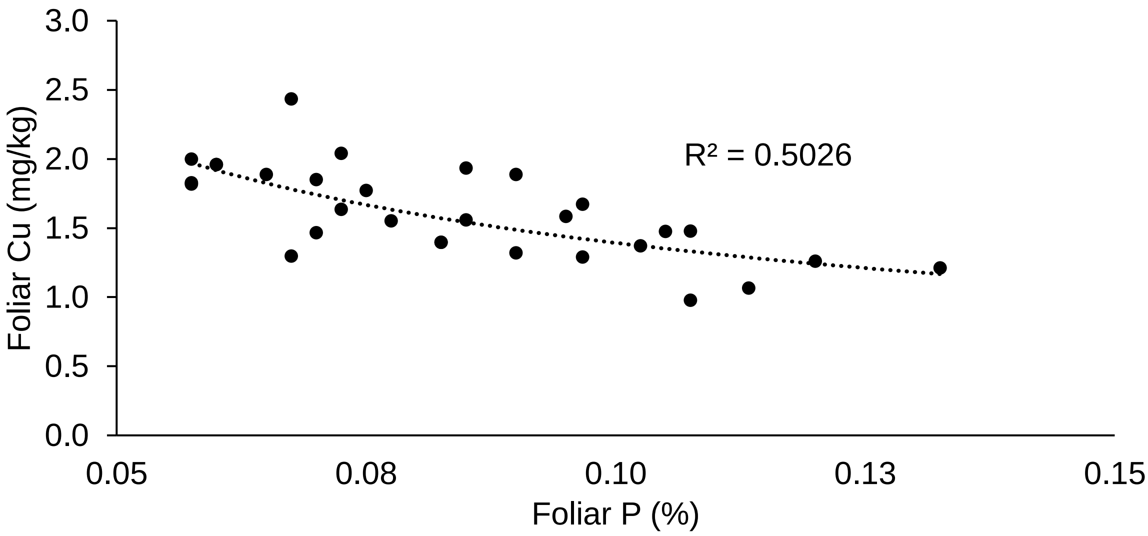

Figure 3.27. Correlation between foliar Cu and foliar P in the N × P and K × NP experiments.

3.3.3.9. Foliar Zn

P application in the N × P experiment had a significant effect (p=0.009) on foliar Zn concentration; increases from 5.77 – 6.77 mg/kg Zn at 0 – 30 mg/kg P application to 7.32 – 7.45 mg/kg Zn at 45 – 60 mg/kg P application were observed (Figure 3.28). Interestingly, there was no correlation between plant-available P at either 0 – 30 or 20 – 40 cm and Zn, and only a weak, positive correlation (R^2 = 0.3469) was found between foliar P and Zn.

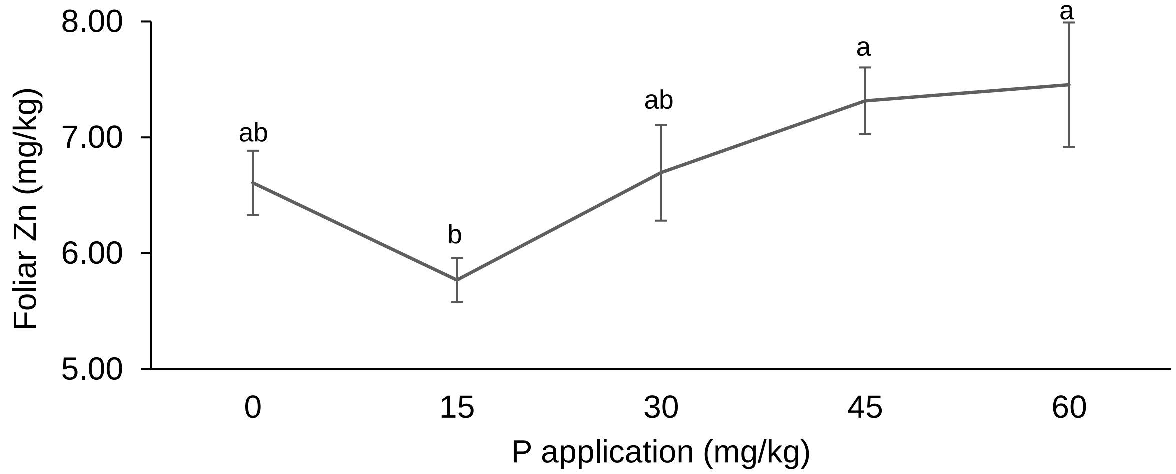

Figure 3.28. The effect of P application on foliar Zn content in the N × P experiment. Different letters indicate significant differences between treatments.

The application of N in the N × P experiment also had a significant effect (p=0.031) on foliar Zn, although no significant interactive effect between N and P application was found (p=0.123). Foliar Zn remained at 6.90 – 7.19 mg/kg at N application rates of 0 – 40 mg/kg and only significantly decreased to 5.90 at an application rate of 60 mg/kg N (Figure 3.29).

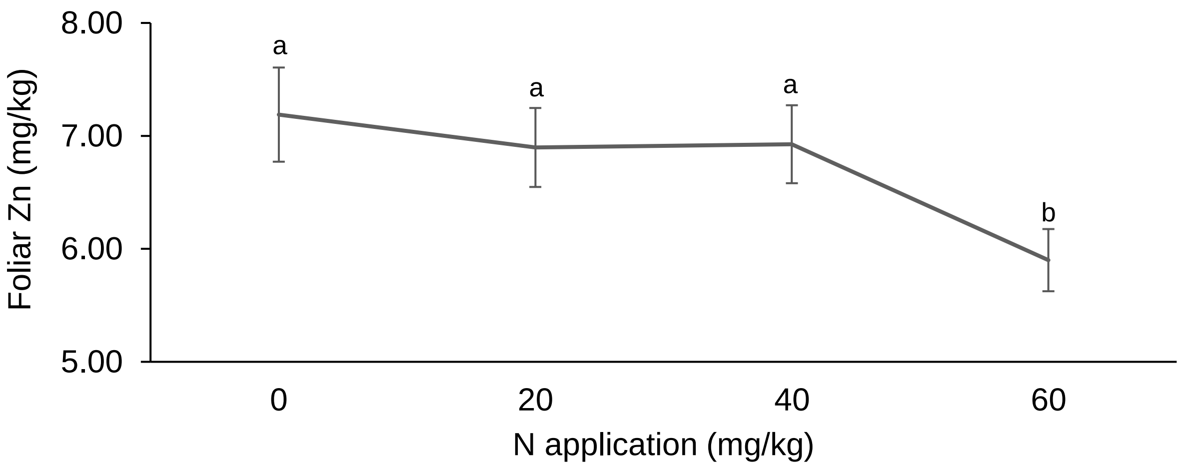

Figure 3.29. The effect of N application on foliar Zn content in the N × P experiment. Different letters indicate significant differences between treatments.

While the application of K alone had no effect on foliar Zn in the K × NP experiment, the application of N in combination with P significantly increased foliar Zn concentration (p=0.001) (Figure 3.30). Furthermore, there was a significant (p=0.011) interactive effect between the application of K and the application of N and P. The application of 20 mg/kg N and 30 mg/kg P at 40 mg/kg K increased foliar Zn from 5.75 mg/kg to 9.35 mg/kg at the same K application rate. A strong Zn – P antagonism is known to exist in most crops. A decreasing trend in foliar Zn was observed when chicken litter compost was applied to rooibos plants, and negative correlation between plant-available Zn and soil P was observed by Smith (2014). The antagonism was not found in this study, due to the Zn content in the TSP fertiliser used.

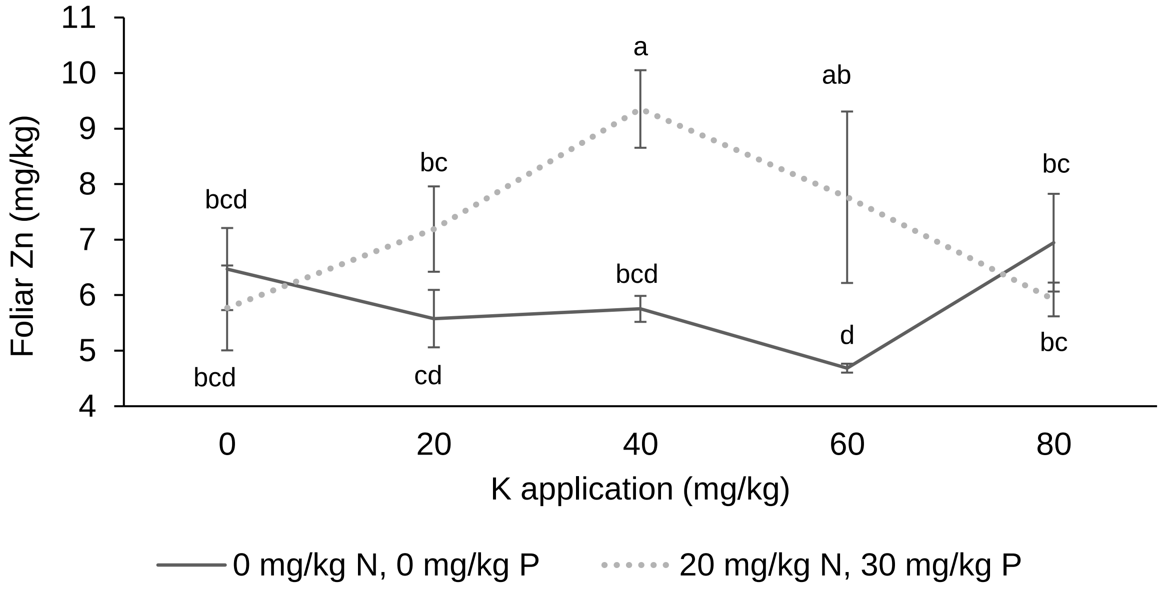

Figure 3.30. The effect of K application with and without the application of N and P on foliar Zn content in the K × NP experiment. Different letters indicate significant differences between all treatments in both data series.

3.3.3.10. Foliar Mn

Foliar Mn levels were not affected by the treatments applied in this study. Mn concentration ranged from 81.3 – 161 mg/kg in the the N × P experiment and 52.9 – 108 mg/kg in the K × NP experiment. Mean concentrations were 105 ± 20.9 mg/kg and 79.5 ± 15.5 mg/kg respectively. Mn is known to play an important role in oxidation-reduction processes in photosynthesis, and chloroplasts quickly display signs of deterioration when Mn supply is insufficient (Kabata-Pendias, 2011). Foliar Mn values in this study are lower than those found by Smith (2014) in the same area, where one-year old plants had an average foliar Mn concentration of 161.83 mg/kg. A clear negative correlation (R^2=0.5439) with foliar K was also found possibly indicating a tissue dilution effect, as K-fertilised plants grew larger, alternatively the negative correlation between foliar Mn and foliar K may be due to an antagonism (Figure 3.31).

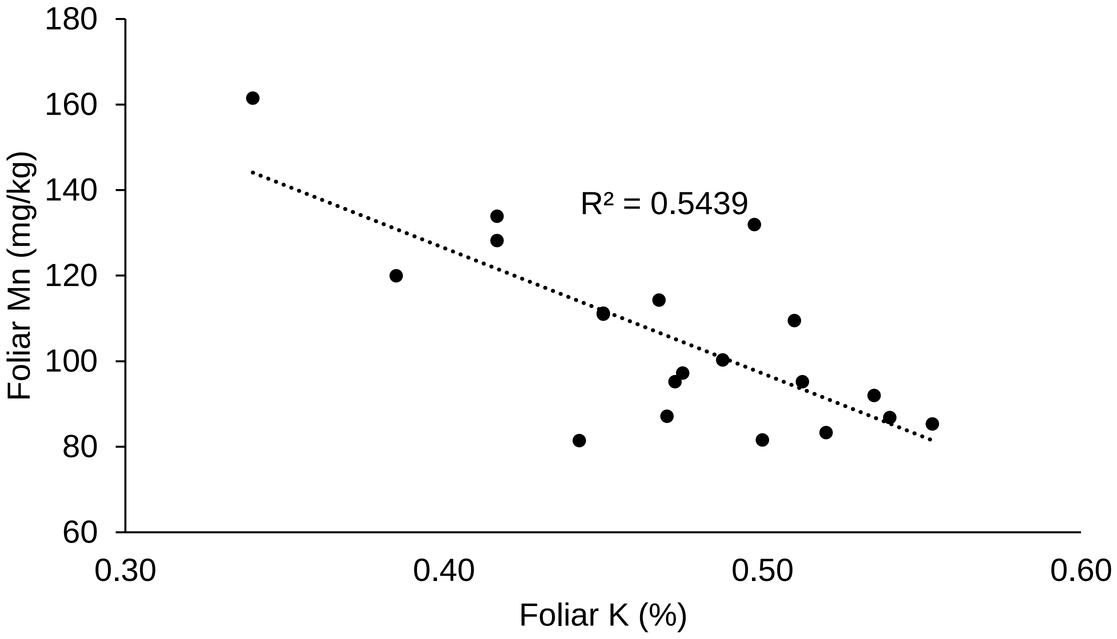

Figure 3.31. Correlation between foliar Mn and foliar K in the N x P experiment.

3.3.3.11. Foliar B

P application in the N × P experiment had a significant effect (p=0.048) on foliar B concentration. P application had an interactive effect with N application (p=0.027) on foliar B, although there was no clear relationship between foliar B concentration and N application. A general decreasing trend in foliar B was observed as P application was increased. Boron concentration declined from an average of 66.3 mg/kg at 0 mg/kg P applied to 57.5 mg/kg at the 60 mg/kg P applied, although there were no significant differences in concentration between 0 – 40 mg/kg P (Figure 3.32). The negative effect of P application on foliar B concentration can be ascribed to the inhibitory effect of phosphate ions on B uptake and mobility (Kabata-Pendias, 2011). No treatment effect was observed in the K × NP experiment, presumably because of the lower P application (30 mg/kg). Average foliar B concentration ranged between 52.4 and 66.7 mg/kg with a mean of 60.3 ± 4.92 mg/kg. All treatments in this study were somewhat higher than the mean of 28.35 mg/kg in one-year old plants in the same area found by Smith (2014). Boron is essential for the synthesis and translocation of carbohydrates, the formation of cellular membranes and reproductive processes in plants (Kabata-Pendias, 2011) and it is assumed that levels were adequate since no correlations between foliar B and any other plant or soil parameters were found.

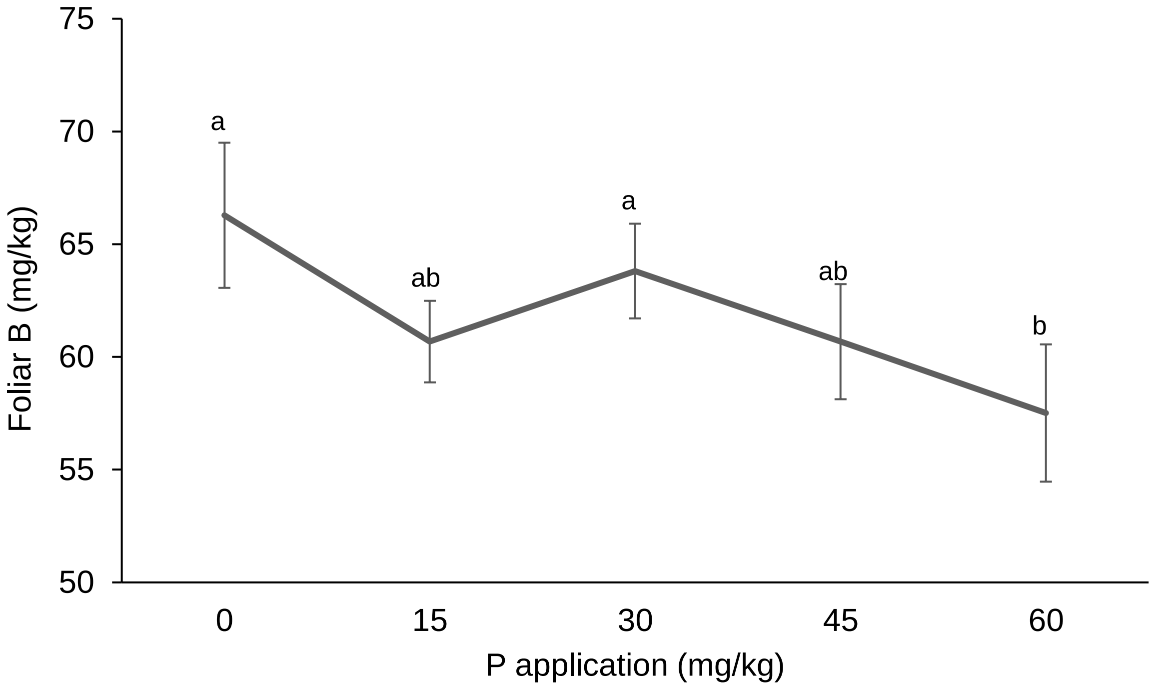

Figure 3.32. The effect of P application on foliar B content in the N × P experiment. Different letters indicate significant differences between treatments.

3.3.3.12. Foliar Al

No treatment effects on foliar Al were found in this study. Concentrations ranged from 256 – 362 mg/kg with a mean of 290 ± 27.9 mg/kg in the N × P experiment and 227 – 280 mg/kg with a mean of 256 ± 16.3 mg/kg in the K × NP experiment. These values correspond well to those of Smith (2014). Al concentrations in rooibos plants tend to be higher than those in other commonly cultivated crops, for example wheat (31 mg/kg) but its concentration in plants appears to vary as a function of soil concentration and biotic factors (Kabata-Pendias, 2011). Not much is known about the role of Al in plant physiology, although there is evidence of its benefit to plants tolerant to high concentrations, when supplied in combination with other nutrients (Watanabe et al., 2005). No correlations between Al and other plant and soil parameters were found in this study.

3.3.4. Plant analyses

3.3.4.1. Plant height

Rooibos plant material is genetically very heterogeneous as it is mainly grown from wild-harvested seed, and no selective breeding programmes have ever been implemented. Thus it is often difficult to obtain statistically significant differences in plant biomass measurements, however, trends in the data can be observed. Four months after planting (October 2016), maximum plant height (25 cm) was attained with a P application of 30 mg/kg (Figure 3.33). However, no statistically significant differences in plant height between treatments were found in the N × P experiment at four months. Plant height ranged from 17.9 – 26.9 cm, with a mean of 22.4 ± 2.41 cm. Eight months after planting, the application of N (Figure 3.34) and P had no significant effect. Maximum plant height was obtained at 15 mg/kg P (62.0 cm), whereas the higher P application rates decreased plant height from 60.0 – 63.0 to 54.6 – 56.4 cm (Figure 3.33). At 12 months after planting, plant height was also significantly affected by P (p=0.0062), but not N application. A clear suppressing effect of P application on plant height was observed where P application of 30 – 60 mg/kg was associated with a decrease in plant height from 72.3 – 77.5 cm to 66.3 – 68.7 cm (Figure 3.33). Maximum plant height was obtained (77.5 cm) in the control treatment in this month.

In the K × NP experiment, neither K application alone nor K with 20 mg/kg N and 30 mg/kg P had statistically significant effects on plant height at four, eight or twelve months after planting (Figure 3.35). Four months after planting, maximum plant height (29.8 cm) was obtained in K applications of 20 mg/kg without N and P, and 40 mg/kg K with N and P (29.6 cm), compared to the control (22.6 cm) (Figure 3.35a). After eight months, maximum plant height (72.6 cm) was attained with the application of 20 mg/kg K alone (Figure 3.35b). The addition of N and P in combination with K significantly (p= 0.012) reduced plant height compared to the treatments in which K was applied alone (Figure 3.35b) from an average height of 67.5 cm to 54.9 cm, likely due to the suppressing effect of P (Figure 3.33b). At twelve months, maximum plant height (82.8 – 84.6 cm) was observed at K applications of 0 – 40 mg/kg and reduced by K applications over 40 mg/kg, and at all rates of K application when applied in combination with N and P, reducing average height from 81.5 to 64.0 cm (p=0.0107) (Figure 3.35c). At four, eight and twelve months, the application of 80 mg/kg K with N and P caused the greatest reduction in height, relative to the control treatment (Figure 3.35). Overall, the greatest height observed in this study was 83.8 – 84.6 cm at twelve months, obtained by the application of 20 – 40 mg/kg K. Correlations between height at 8 and 12 months and plant-available P and foliar P in both the N x P and the K x NP experiments were low (R^2=0.4000) and it is thus likely that at soil P applications higher than 30 mg/kg plant growth was stunted and decreased P uptake occurred after Oct 2016, when plants began to be subjected to more abiotic stress such as decreased rainfall (Figure 3.36), low humidity, wind and greater solar radiation. This is clearly illustrated in Figure 3.33-3.35, where maximum height was observed at lower fertiliser applications as the growing season progressed and water stress increased.

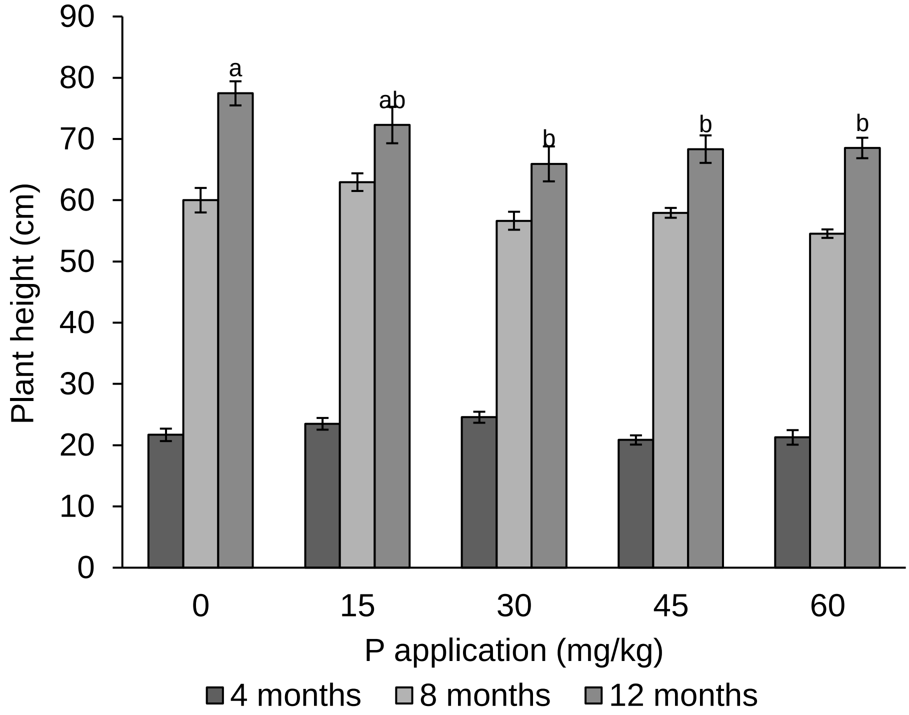

Figure 3.33. The effect of P application on plant height 4, 8 and 12 months after planting in the N × P experiment.

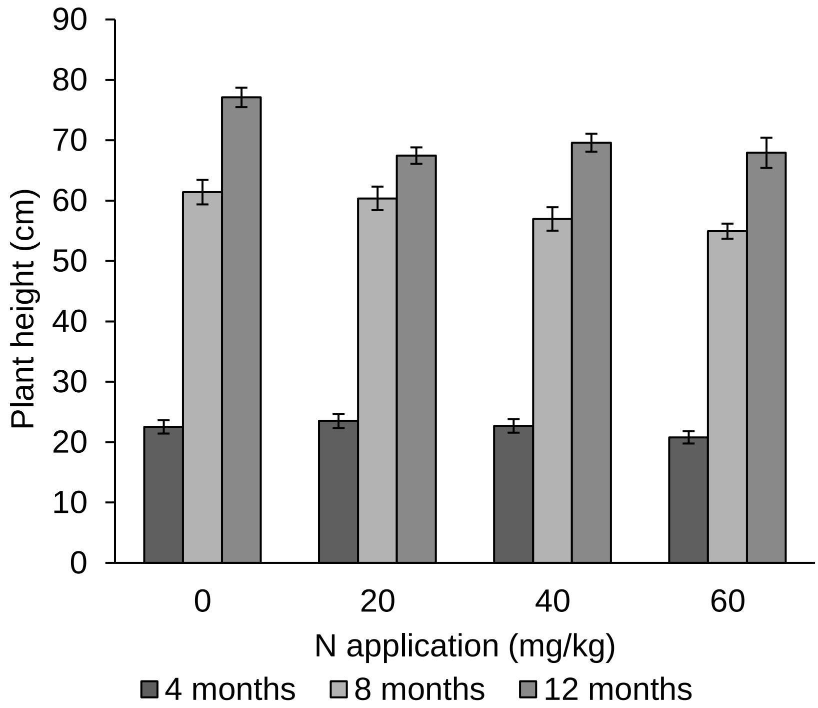

Figure 3.34.The effect on N application on plant height 4, 8 and 12 months after planting in the N x P experiment.

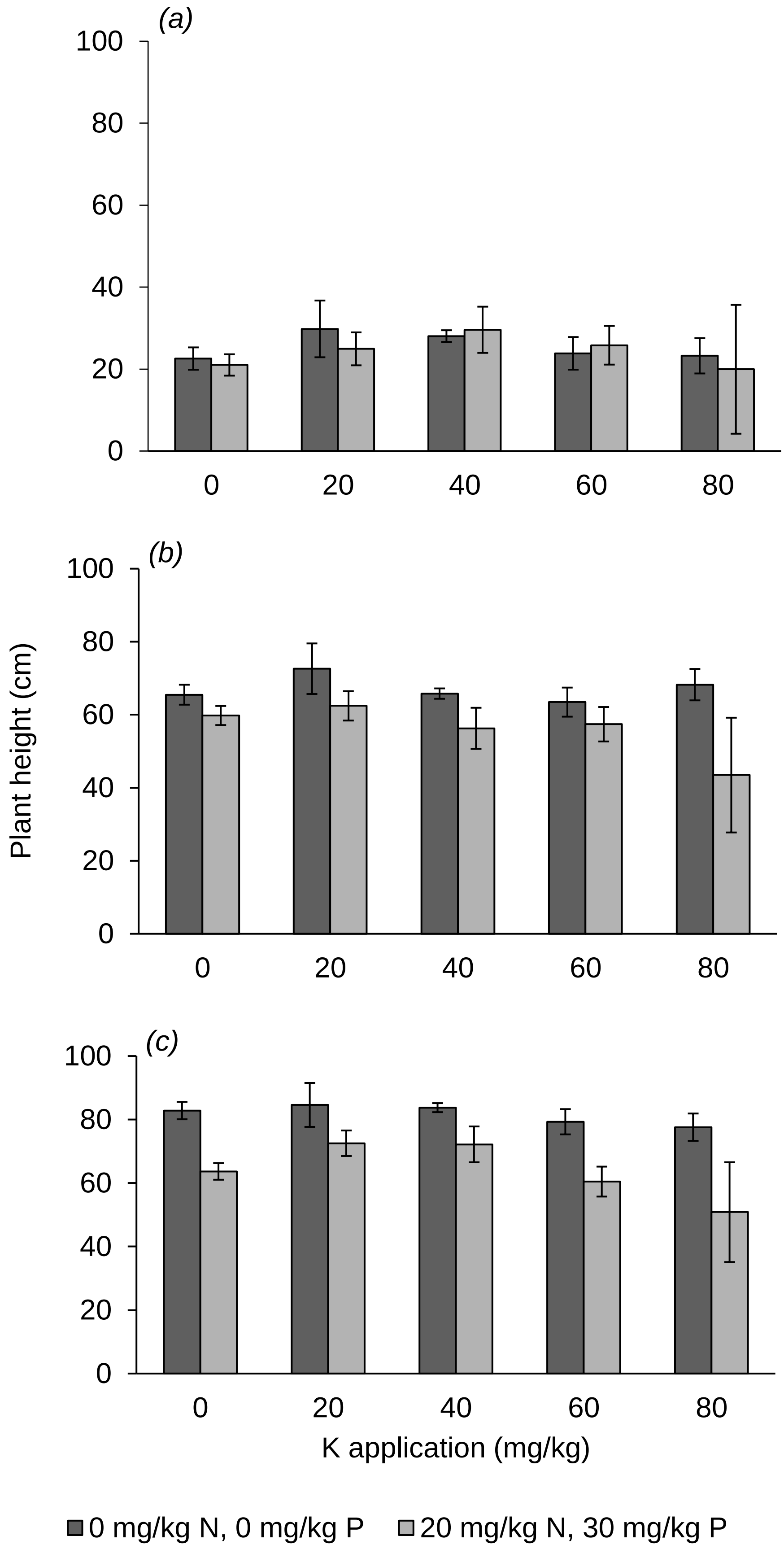

Figure 3.35. The effect on K with and without 20 mg/kg N and 30 mg/kg P height at (a) 4, (b) 8 and (c) 12 months after planting in the K × NP experiment.

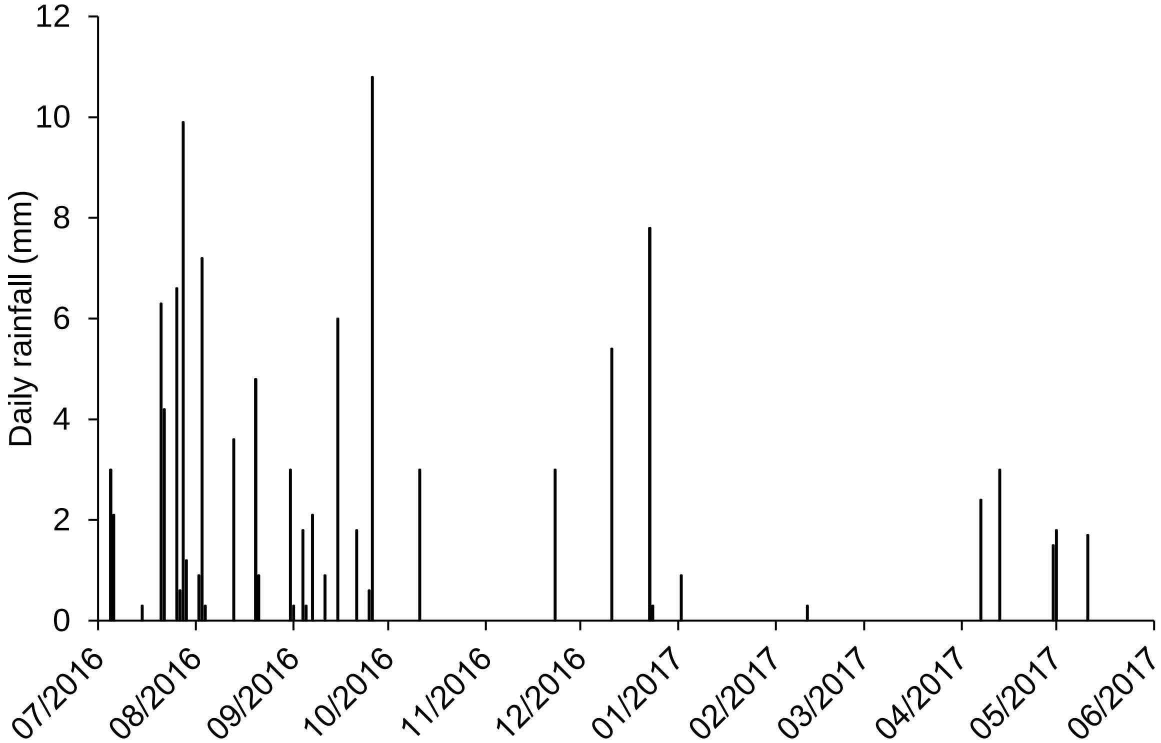

Figure 3.36. Graph of daily rainfall at the field trial site from 1 July 2016 to 31 May 2017.

3.3.4.2. Plant survival

Plant survival at 12 months was significantly affected ($p<0.001$) by N application in the N × P experiment, although no relationship between foliar N and survival was found. Survival decreased from an average of 30.4 % at an N application of 0 – 40 mg/kg to 19.2 % at an application rate of 60 mg/kg (Figure 3.37). However, no correlation was found between foliar N and plant survival.

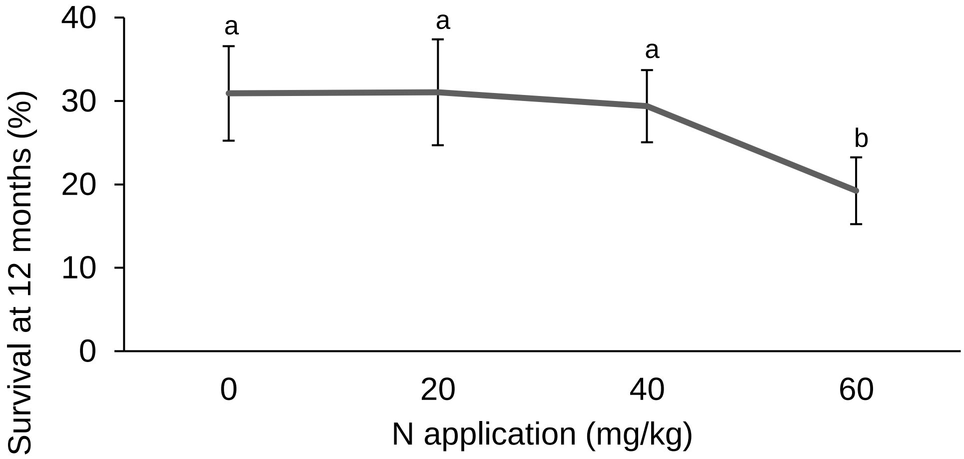

Figure 3.37. The effect N application on plant survival after one year in the N × P experiment.

P application also had a highly significant effect on plant survival at 12 months ($p<0.001$) in this experiment. A linear trend of decreasing survival with an increase in P application was observed. Survival decreased significantly from 46.8 % in the control to 15.4 – 17.9 % at application rate of 45 - 60 mg/kg P (Figure 3.38).

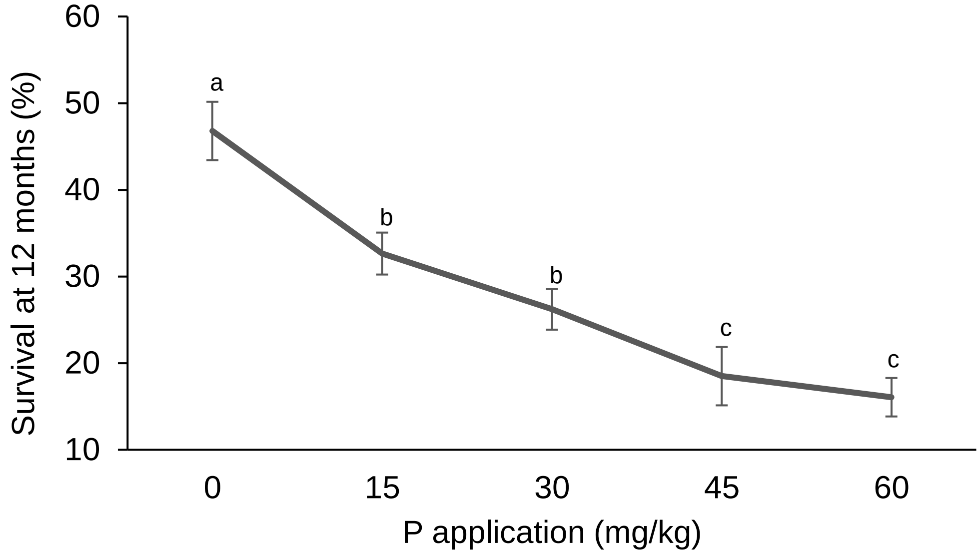

Figure 3.38. The effect P application on plant survival after one year in the N × P experiment. Different letters indicate significant differences between treatments.

Furthermore, Pearson and Spearman tests indicated a significant negative correlation between survival one year after planting and plant available P at 20 – 40 cm ($p<0.01$) and foliar P ($p<0.010$) in the N × P experiment (Table 3.6). Correlation coefficients (R^2) between survival and foliar P and plant-available soil P (20 – 40 cm) were 0.4320 and 0.6676 respectively. Figure 3.39 and Figure 3.40 illustrate the decrease in plant survival after one year associated with higher foliar P and soil P levels. Survival was observed to decrease below 40 % at foliar P levels higher than 0.08% and at soil P levels higher than 10 mg/kg. In the K × NP experiment, the application of 20 mg/kg N and 30 mg/kg P significantly lowered survival ($p<0.001$) from 54.5 to 36.4 %. The application of K did not have a significant effect on plant survival, although survival at 20 mg/kg K with N and P was somewhat higher (38.5%) than N and P without K (29.9%) (Figure 3.41).

Table 3.6. Correlations between plant survival and plant and soil parameters in the N × P experiment using Pearson and Spearman correlation tests.

	Pearson		Spearman	
Parameter	Coefficient	P-value	Coefficient	P-value
Soil P (20 – 40 cm)	–0.54	<0.01	–0.58	<0.01
Foliar P	–0.48	<0.01	–0.55	<0.01

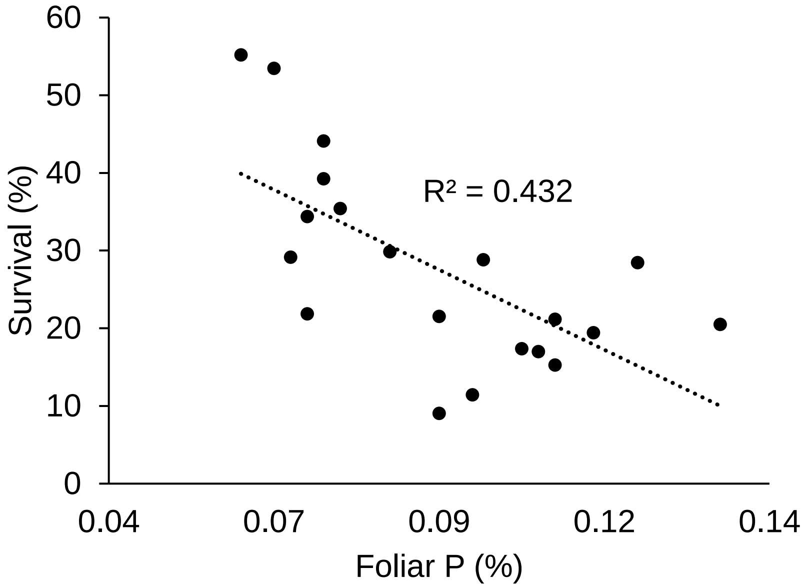

Figure 3.39. Correlation between survival one year after planting and foliar P in N × P experiment

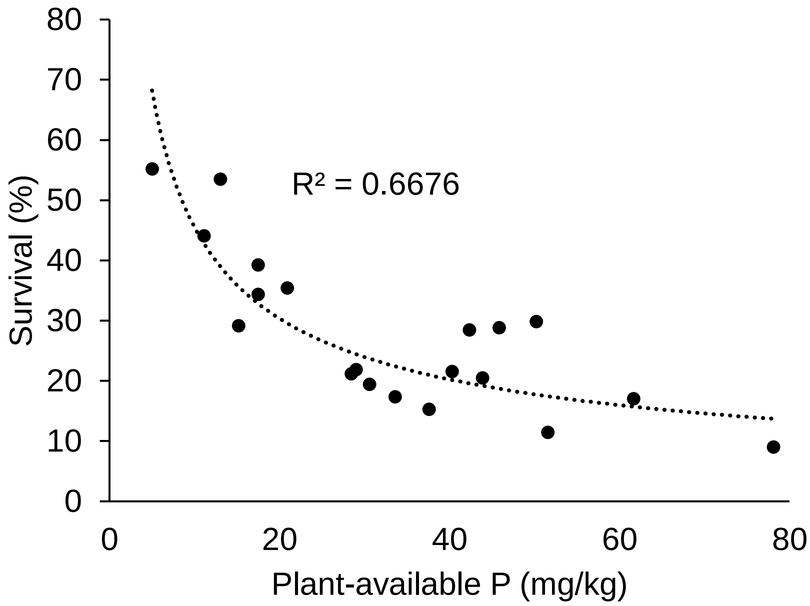

Figure 3.40. Correlation between survival one year after planting and Bray II plant-available P at 20 – 40 cm in the N × P experiment

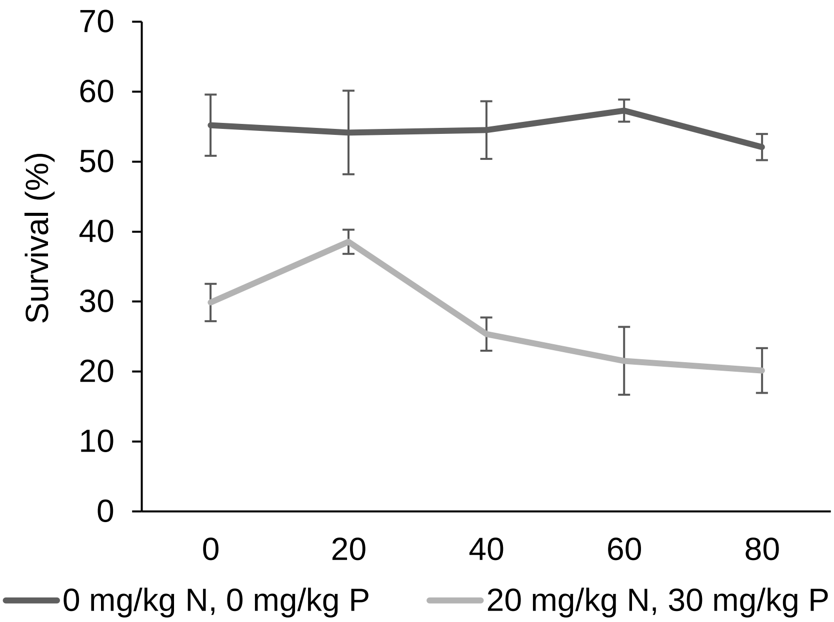

Figure 3.41. The effect of K application with and without 20 mg/kg N and 30 mg/kg P on survival after one year in the K × NP experiment.

Pearson and Spearman correlation tests indicated significant positive correlations ($p<0.01$) between plant survival and pH (H_2O) at 20 – 40 cm and plant height at 8 and 12 months, and significant negative correlations with soil P at 0 – 20 and 20 – 40 cm, foliar P (Table 3.7).

Table 3.7. Correlations between plant survival and plant and soil parameters in the K × NP experiment using Pearson and Spearman correlation tests.

	Pearson		Spearman	
Parameter	Coefficient	P-value	Coefficient	P-value
pH (H_2O) (20 –40 cm)	+0.49	<0.01	+0.55	<0.01
Soil P (0 – 20 cm)	–0.66	<0.01	–0.79	<0.01
Soil P (20 – 40 cm)	–0.67	<0.01	–0.76	<0.01
Foliar P	–0.78	<0.01	–0.79	<0.01
Plant height 8 months	+0.49	<0.01	+0.54	<0.01
Plant height 12 months	+0.52	<0.01	+0.50	<0.01

The correlation between survival and plant-available P in the K × NP experiment was higher (R^2 = 0.8158) at 20 – 40 cm than at 0 – 20 cm (R^2 =0.6454), probably due to the depth to which the fertiliser was ploughed. Figure 3.42 illustrates the clear negative relationship between soil P at 20 – 40 cm and survival, where plant survival decreased below 50 % at plant-available P levels higher than 20 mg/kg P. The relationship between survival and soil P in this experiment was also reflected in foliar P concentration, with a strong negative correlation between survival and foliar P concentration (R^2 = 0.8008). An increase in Foliar P concentration of 0.06 to 0.10 % decreased plant survival dramatically from over 50 % to approximately 20 % (Figure 3.43).

The correlation between survival and plant-available P the K × NP experiment was higher (R^2 = 0.8158) at 20 – 40 cm than at 0 – 20 cm (R^2 =0.6454), probably due to the depth to which the fertiliser was ploughed. Figure 3.42 illustrates the clear negative relationship between soil P at 20 – 40 cm and survival, where plant survival decreased below 50 % at plant-available P levels higher than 20 mg/kg P. The relationship between survival and soil P in this experiment was also reflected in foliar P concentration, with a strong negative correlation between survival and foliar P concentration (R^2 = 0.8008). An increase in Foliar P concentration of 0.06 to 0.10 % decreased plant survival dramatically from over 50 % to approximately 20 % (Figure 3.43).

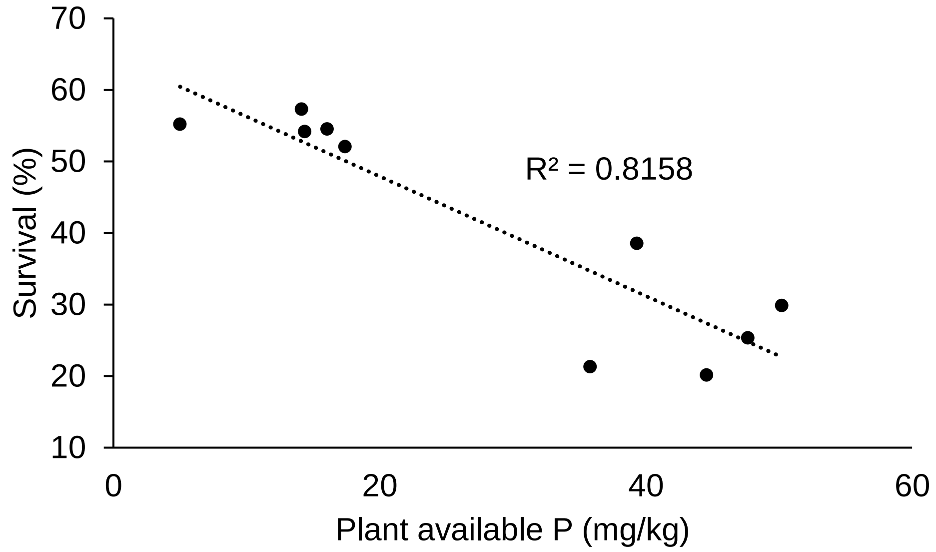

Figure 3.42. Linear correlation between plant survival after one year and Bray II plant-available P at 20 – 40 cm in the K × NP experiment.

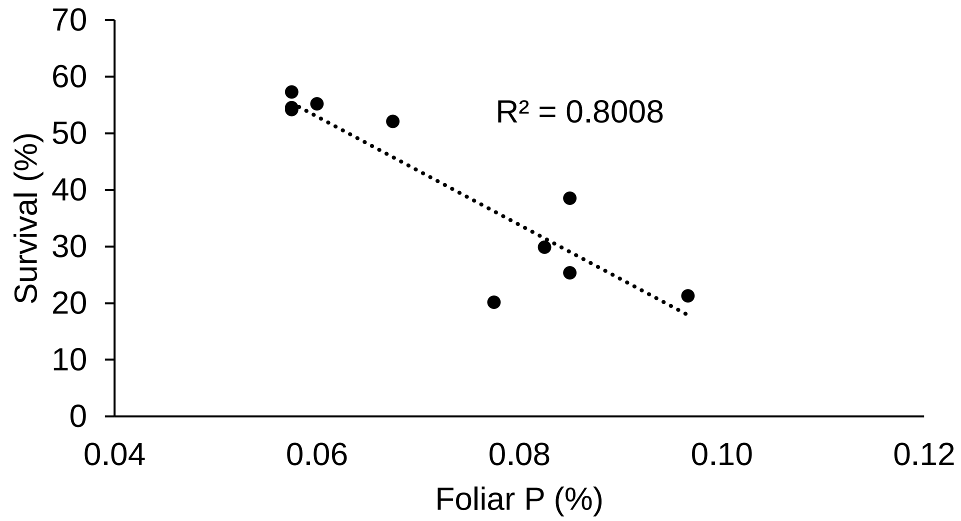

Figure 3.43. Plant survival one year after planting and foliar P in the K × NP experiment.

The positive correlation (R^2=0.5875) between survival and soil pH at 20 – 40 cm (Figure 3.44) can be explained by the decrease in pH caused by the application of 20 mg/kg N and 30 mg/kg P (Figure 3.4). Rooibos plants fynbos species possess poor P-uptake regulation, and even at a low application rate of 15 mg/kg, a significant decrease in plant survival was observed, decreasing from 46.8 to 31.4 % (Figure 3.38). A positive correlation was found between survival and plant height at eight months (R^2=0.7021) and at 12 months (R^2=0.8128) (Figure 3.45) in the K × NP experiment. P application and resultant higher foliar P was shown to be associated with lower plant height (Figure 3.39 and Figure 3.40) and survival (Figure 3.42 and Figure 3.43). If plant height can be assumed as being a rough indicator of vigour, plants that were more vigorous due to lower soil P and foliar P levels were more likely to survive after one year in the field. Plant height is less likely to be a reliable indicator of vigour as plants mature and become bushier.

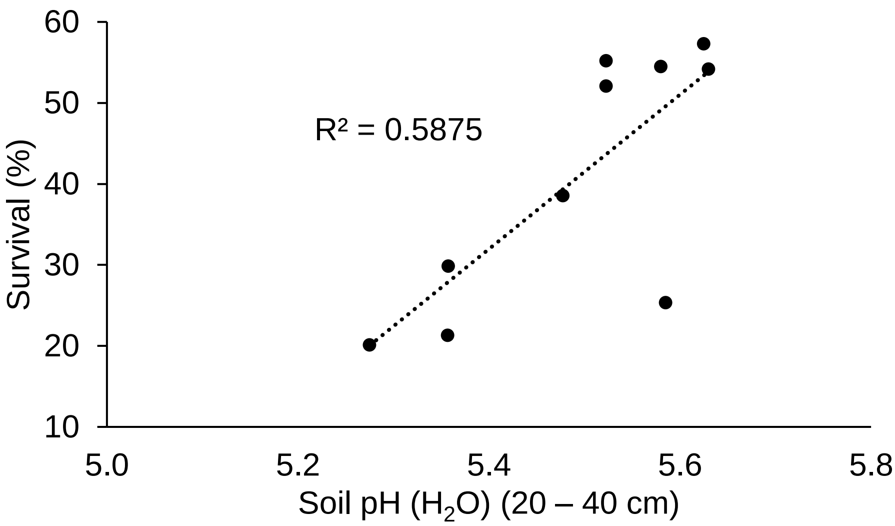

Figure 3.44. Correlation between plant survival after one year and soil pH (H_2O) at 20 – 40 cm in the K × NP experiment.

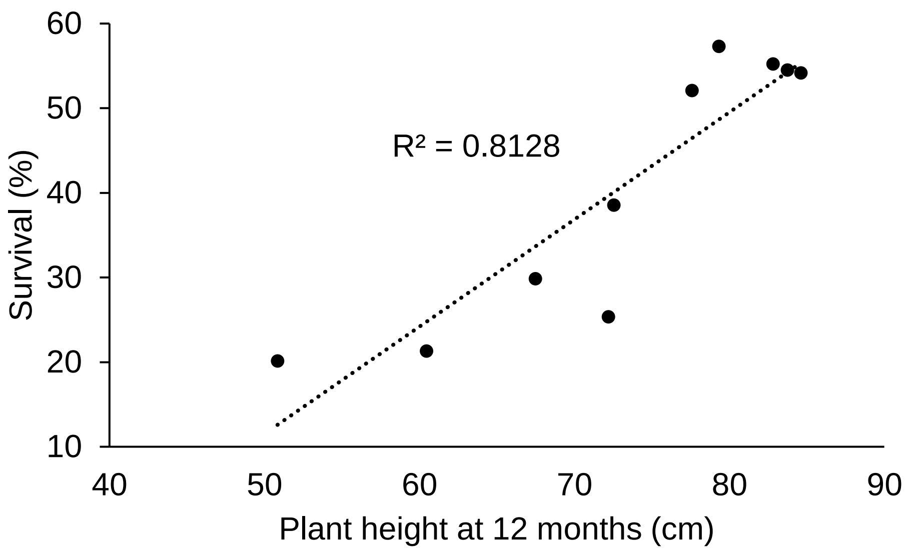

Figure 3.45. Correlation between plant survival after one year and plant height at one year in the K × NP experiment.

3.3.4.3. Biomass

Neither N nor P application had a statistically significant effect on plant above-ground biomass in the N × P experiment. Similar to other plant data, no significant interactive effect between N and P application was found. However, a clear trend was observed in biomass response to applied N and P. The application of 20 mg/kg N increased plant biomass to 54.8 g/plant, an increase of 21% over the control (45.2 g/plant) and higher than the 40 – 60 mg/kg N applications (41.6 – 44.24 g/plant) (Figure 3.46). A general decrease in biomass occurred with increasing P application, from 57.4 g/plant in the control to 39.6 g/plant in the 60 mg/kg P treatment (Figure 3.46). These results are interesting as the rooibos plant is a legume, yet it seems to benefit from relatively low N applications (20 mg/kg). It is important to note that these above-ground biomass yields do not take plant survival into account.

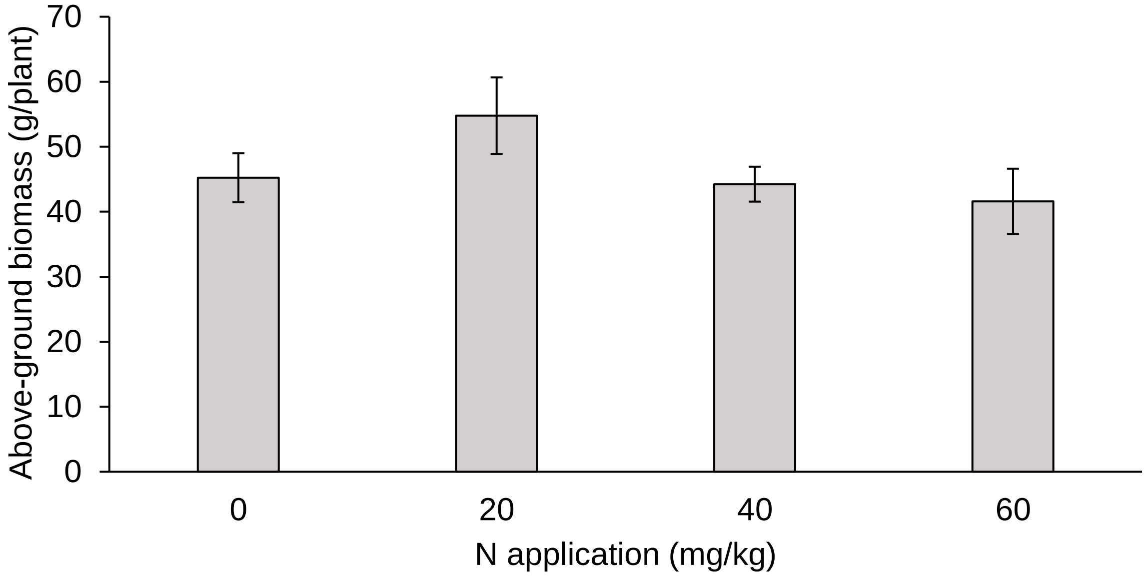

Figure 3.46. The effect of N on above ground biomass in the N × P experiment.

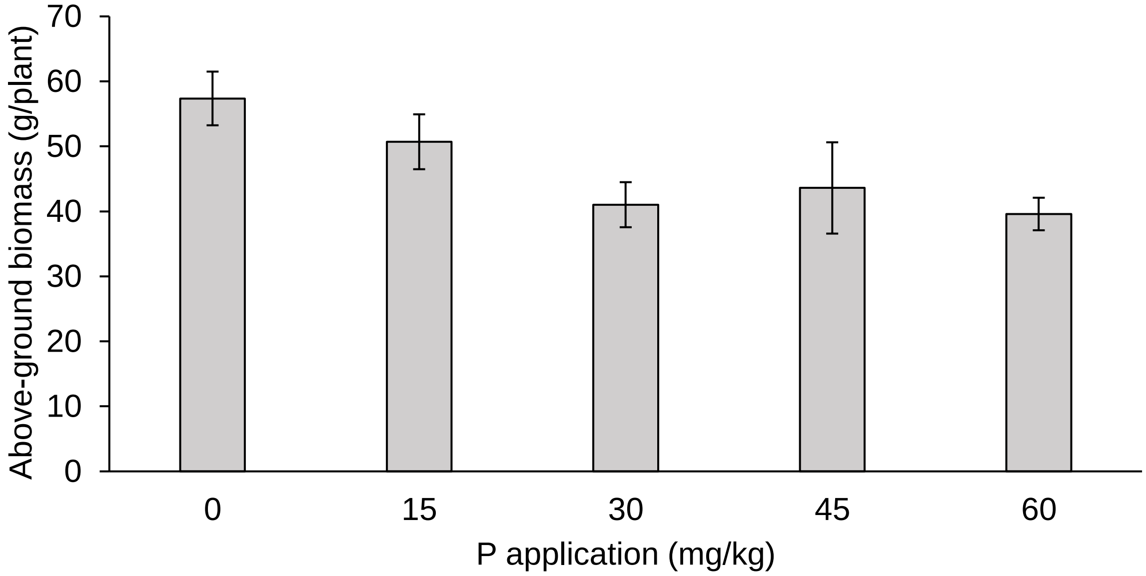

Figure 3.47.The effect of P on above ground biomass in the N × P experiment.

In the K × NP experiment no statistically significant effects or treatment differences were found, however, trends were observed. The highest biomass values in this study were obtained at 20 – 60 mg/kg K application without added N or P (Figure 3.48), compared to the control or 0 – 80 mg/kg K with 20 mg/kg N and 30 mg/kg P (Figure 3.48). Pearson and Spearman tests indicated significant ($p<0.01$) negative correlations between above-ground biomass and foliar P and Ca in the K × NP experiment (Table 3.8). Above-ground biomass and Foliar P were moderately negatively correlated (R^2=0.5929) (Figure 3.49). A negative correlation between above-ground biomass and Foliar Ca was found (R^2=0.5072) (Figure 3.50). This could indicate higher uptake of Ca due to P fertilisation with TSP, and is unlikely to be a toxicity effect.

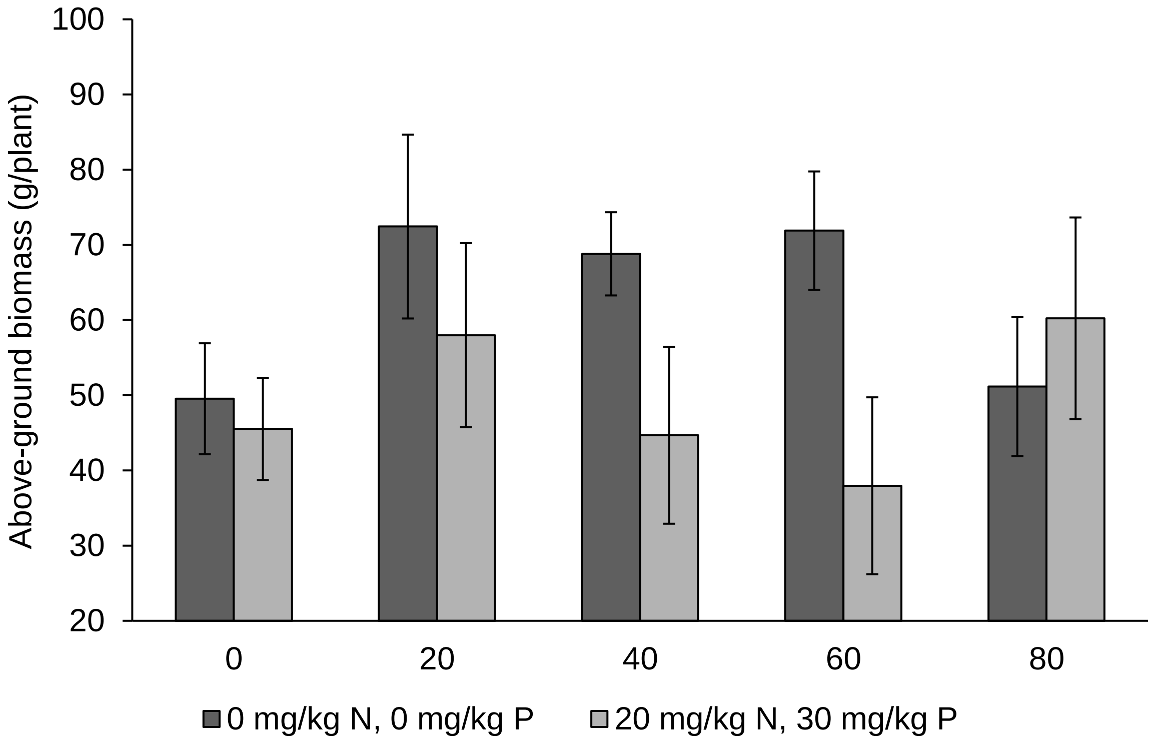

Figure 3.48. The effect of K application with and without 20 mg/kg N and 30 mg/kg P on above ground biomass in the K × NP experiment.

Table 3.8. Correlations between plant biomass and plant and soil parameters in the K × NP experiment using Pearson and Spearman correlation tests.

	Pearson		Spearman	
Parameter	Coefficient	P-value	Coefficient	P-value
Plant P	–0.48	<0.01	–0.48	<0.01
Plant Ca	–0.63	<0.01	–0.58	<0.01

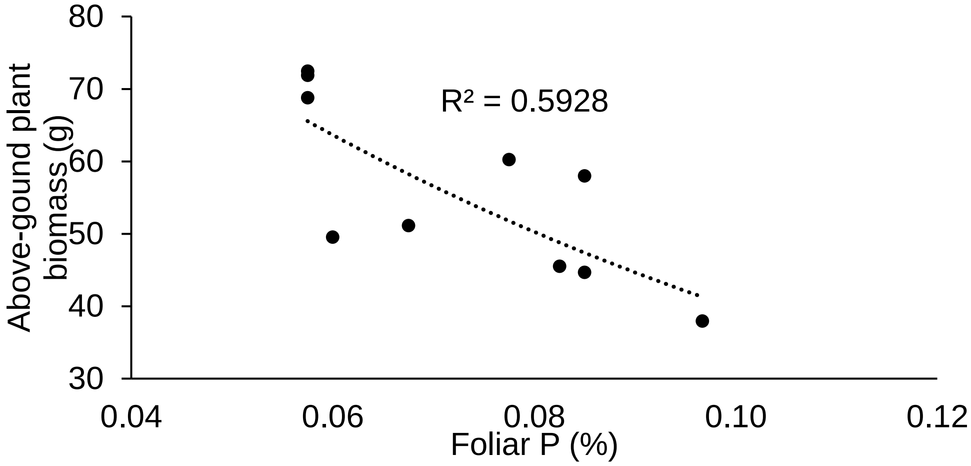

Figure 3.49. Correlation between above-ground biomass and foliar P in the K × NP experiment.

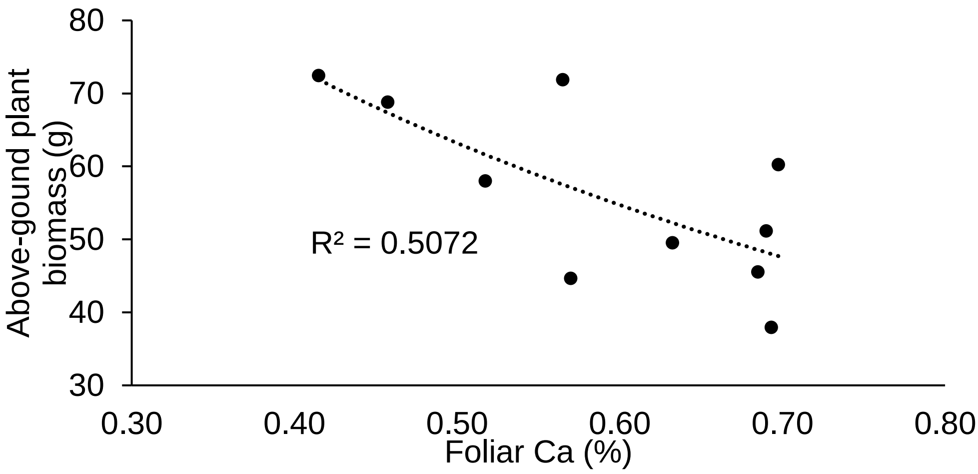

Figure 3.50. Correlation between above-ground biomass and foliar Ca in the K × NP experiment.

The results of this study support those of (Joubert et al., 1987), who found that the ideal plant-available P levels for rooibos seedling growth in the greenhouse up to 3 months were relatively low levels of 15 – 20 mg/kg Bray II P. Drought stress has a significant effect on biomass accumulation in rooibos plants, favouring the proportional increase in root biomass at the expense of above-ground biomass (Lotter et al., 2014) and a lack of water prevents plants from taking up applied nutrients. These results highlight the importance of field trials if fertilisation norms for agricultural purposes are to be found. Greenhouse trials under controlled conditions and fertilised with nutrient solutions cannot approximate the conditions under which a crop is cultivated in the field.

3.3.4.4. Survival-adjusted yield

N application did not have a statistically significant effect on calculated survival-adjusted yield in the N × P experiment, but at a low application rate of 20 mg/kg, yield increased by 23% from 184 kg/ha to 226 kg/ha (Figure 3.51). The application of P had a highly significant effect ($p<0.001$), decreasing yield from 319 kg/ha to 135 kg/ha with a P application of 30 mg/kg; a decrease of nearly 58 % (Figure 3.52). The addition of a further 30 mg/kg reduced yield to 71.2 kg/ha. This decrease in yield associated with increased P application roughly follows a linear trend (R^2=0.8178). For every mg/kg P added a decrease in yield of approximately 6 kg/ha could be expected.

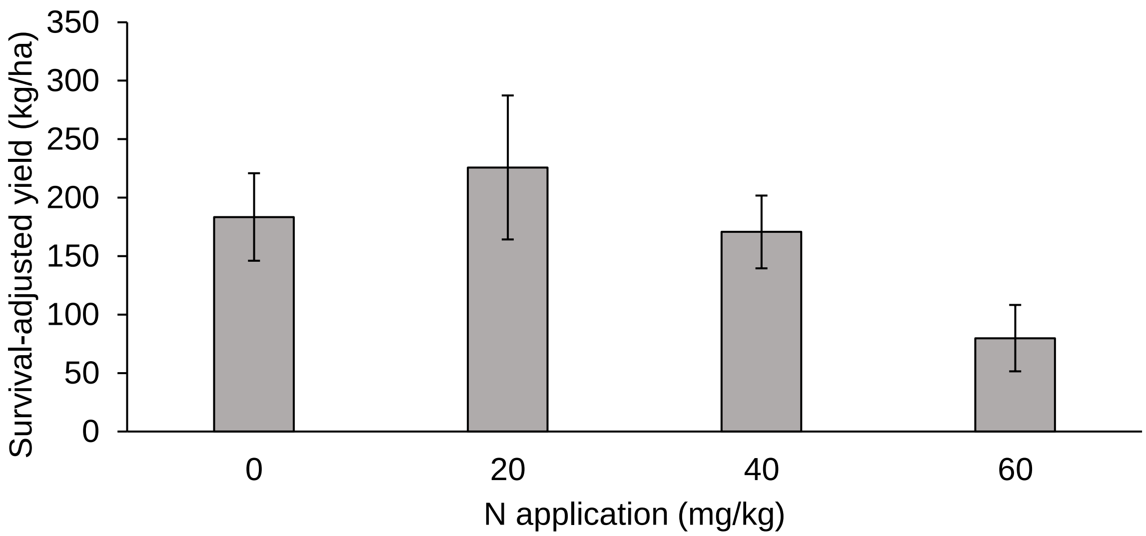

Figure 3.51. Effect of N application on survival-adjusted yield in the N × P experiment.

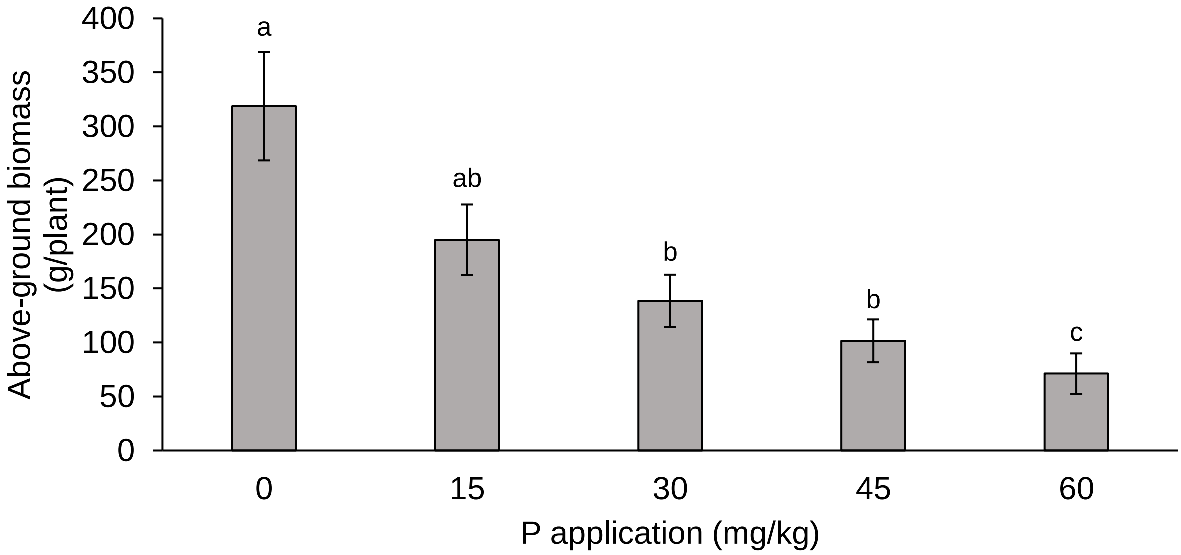

Figure 3.52. Effect of P application on survival-adjusted yield in the N × P experiment. Different letters indicate significant differences between treatments.

Pearson and Spearman tests showed that survival- adjusted yield was significantly negatively correlated with plant - available P at 20 – 40 cm and foliar P (Table 3.9). Significant negative correlations were found between yield and foliar P (R^2=0.5550) (Figure 3.53) and yield and plant-available P (R^2=0.6069) (Figure 3.54).

Table 3.9. Correlations between survival-adjusted yield and plant and soil parameters in the N × P experiment using Pearson and Spearman correlation tests.

	Pearson		Spearman	
Parameter	Coefficient	P-value	Coefficient	P-value
Soil P (20 - 40 cm)	–0.46	<0.01	–0.58	<0.01
Foliar P	–0.39	<0.01	–0.49	<0.01

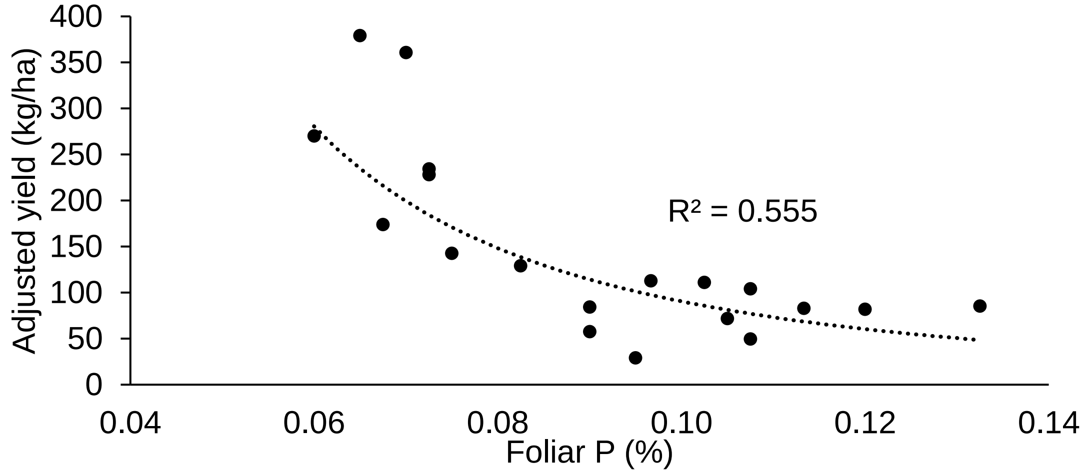

Figure 3.53. Correlation between survival-adjusted yield and foliar P content in the N × P experiment.

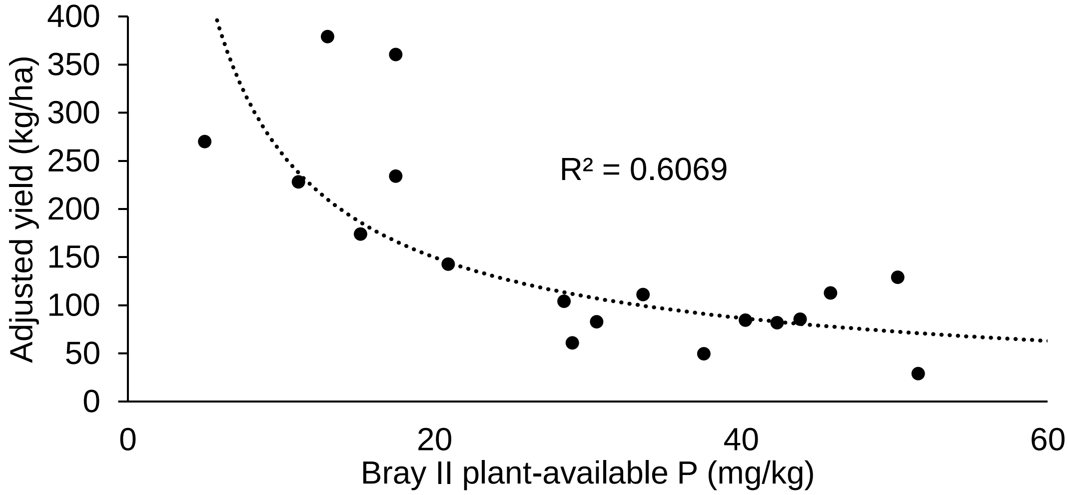

Figure 3.54. Correlation between survival-adjusted yield and plant-available P in the N × P experiment.

The application of K in the K × NP experiment did not have a statistically significant effect on survival-adjusted yield, although a yield of 596.6 kg/ha (the highest observed in this study) was obtained at 20 mg/kg K without the addition of N and P (Figure 3.55). The application of 20 mg/kg and 30 mg/kg, however had a highly significant effect ($p<0.001$) on yield, lowering it from an average of 450.9 (K alone, mean of all treatments) to 164.7 kg/ha (K with N and P, mean of all treatments).

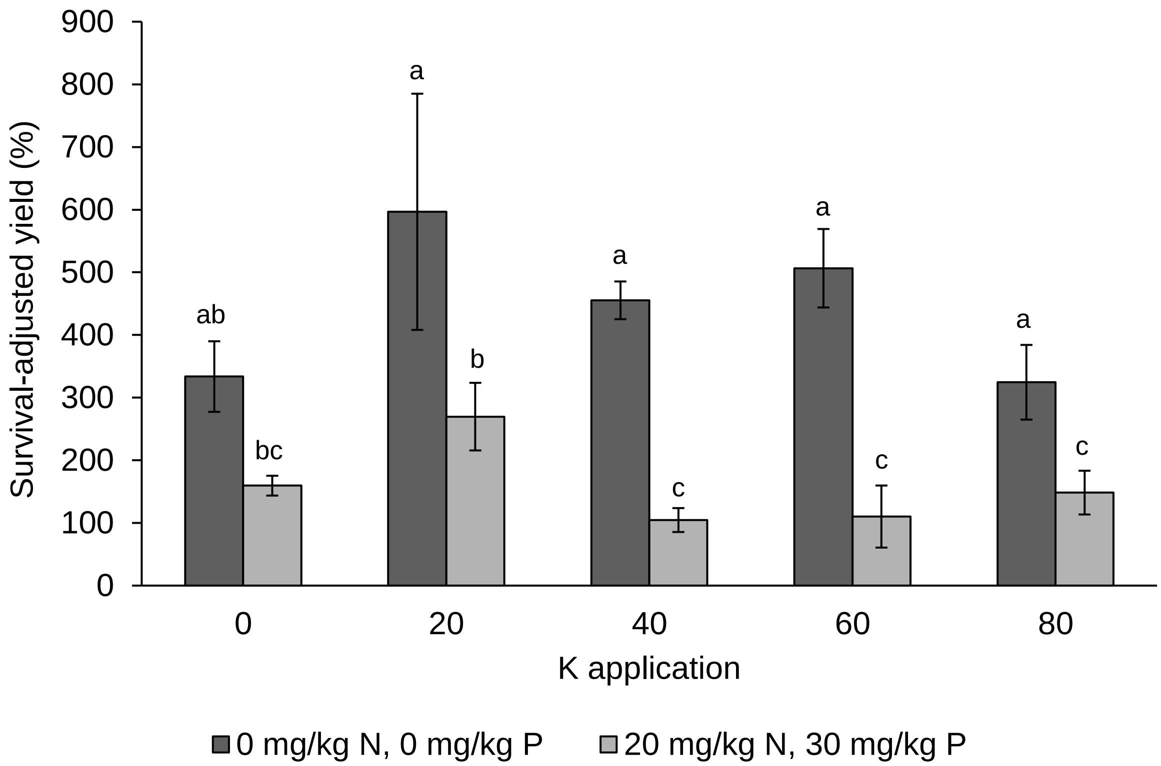

Figure 3.55. Effect of K application with and without 20 mg/kg and 30 mg/kg P on survival-adjusted in the K × NP experiment, Different letters indicate significant differences between treatments.

Pearson and Spearman correlation tests indicated a significant correlation between survival-adjusted yield and pH (H_2O) soil P and foliar P parameters (Table 3.10). No other statistically significant correlations were found.

Table 3.10. Correlations between survival-adjusted yield and plant and soil parameters in the K × NP experiment using Pearson and Spearman correlation tests.

	Pearson		Spearman	
Parameter	Coefficient	P-value	Coefficient	P-value
pH (water)0 - 20	+0.39	0.03	+0.51	<0.01
pH (water) 20 -40	+0.48	<0.01	+0.66	<0.01
Soil P 20 -40	–0.61	<0.01	–0.75	<0.01

Plant P	–0.72	<0.01	–0.81	<0.01

Survival-adjusted yield was negatively correlated (R^2=0.5504) with plant-available P in the K × NP experiment (Figure 3.56). Yield was highest at foliar P concentrations lower than 20 mg/kg but fell below 200 kg/ha at soil P levels higher than 40 mg/kg. Similarly, survival-adjusted yield correlated well (R^2 = 0.8223) with foliar P in the K × NP experiment. Yield declined as foliar P levels increased, falling below 200 kg/ha at foliar P levels higher than 0.08 % (Figure 3.57).

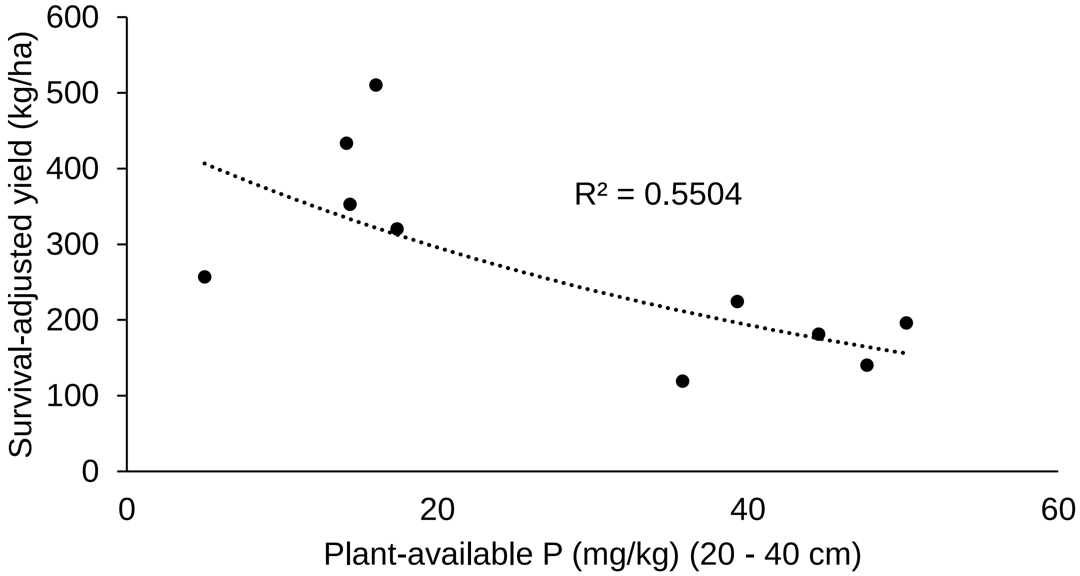

Figure 3.56. Correlation between survival-adjusted yield and plant-available soil P at 20 – 40 cm in the K × NP experiment.

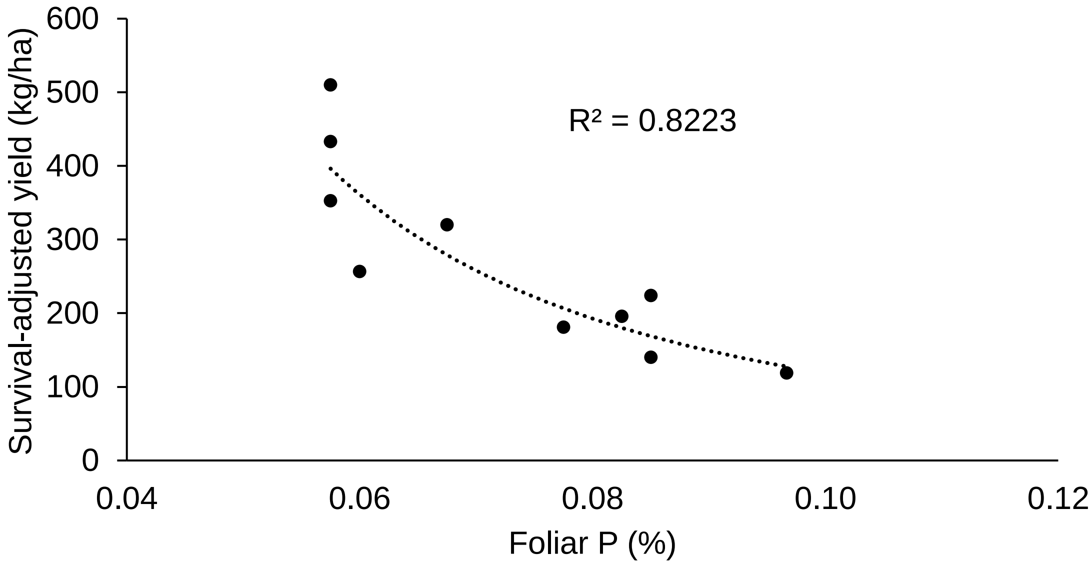

Figure 3.57. Correlation between survival-adjusted yield and foliar P concentration in the K × NP experiment.

3.3.5. Soil enzyme assays

3.3.5.1. Xanthine dehydrogenase and glutamine oxoglutarate aminotransferase

XDH enzyme activity was significantly increased from 0.18 to 0.24 µmol/g soil/min by the combined application of N (p=0.0053) and P (p=0.0100) (Figure 3.58). Both the application of N (p=0.0195) and P (p<0.0001) at low levels significantly affected the activity of GOGAT, where the application of 15 mg/kg P alone depressed the activity of this enzyme from 0.28 in the control treatment to 0.09 µmol/g soil/min, and the application of 20 mg/kg N increased the activity of this enzyme from 0.29 to 0.34 µmol/g soil/min (Figure 3.59). Xanthine dehydrogenase (XDH) and glutamine oxoglutarate aminotransferase (GOGAT) enzymes are involved in the conversion of inorganic nitrogen to ureides and amino acids, respectively. Plants have transporters to take up both these organic forms of N from the soil and they are also important for microbial biomass accumulation. The application of 20 mg/kg N alone increased the synthesis of amino acids. P application of 15 mg/kg depressed GOGAT activity considerably, an indication that its application may lower the rate of amino acid synthesis. Ureide synthesis is a more N-dense form of organic N storage (Valentine et al., 2017). This may benefit the organic N storage of soil microbes for this treatment.

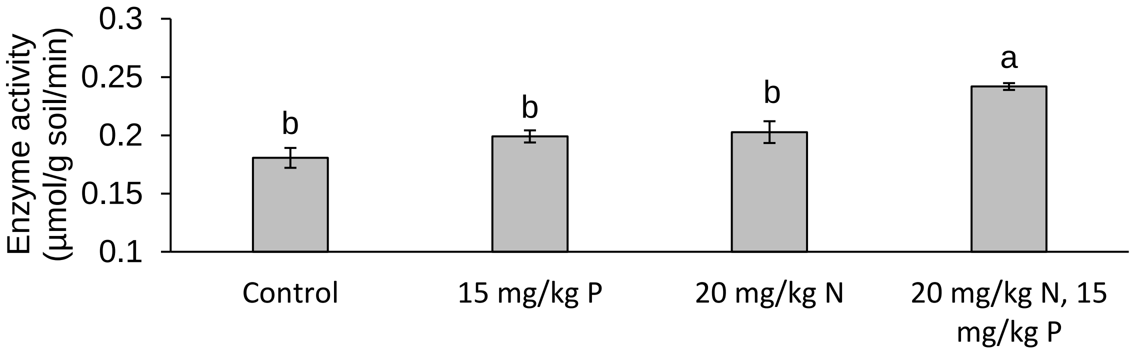

Figure 3.58. XDH enzyme activity in rhizosphere soil in the control, 15 mg/kg P, 20 mg/kg P and 20 mg/kg N, 15 mg/kg P treatments.

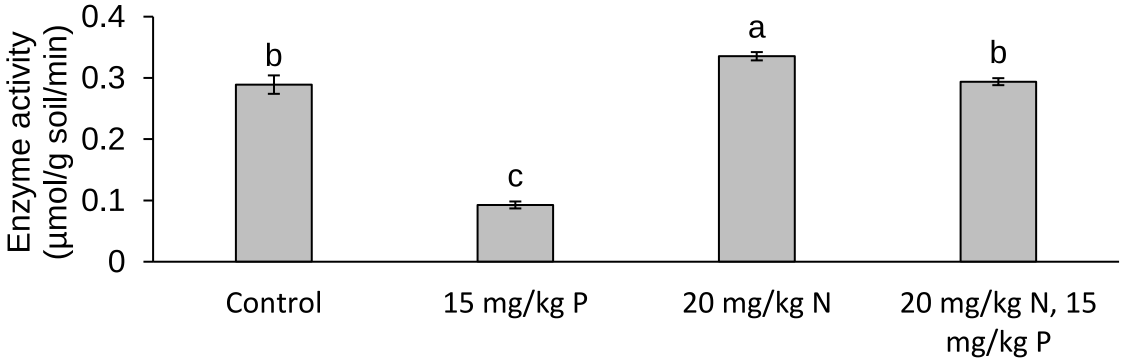

Figure 3.59. GOGAT enzyme activity in rhizosphere soil in the control, 15 mg/kg P, 20 mg/kg P and 20 mg/kg N, 15 mg/kg P treatments.

3.3.5.2. Pyruvate kinase

The application of N had no effect on PK enzyme activity, but the application of 15 mg/kg P significantly increased activity from 1.07 to 1.23 µmol/g soil/min (Figure 3.60). PEP is transformed into pyruvate by PK. This is a reaction which requires ATP and is thus favoured when plants and microbes are supplied with phosphate (Thuynsma et al., 2014 ab).

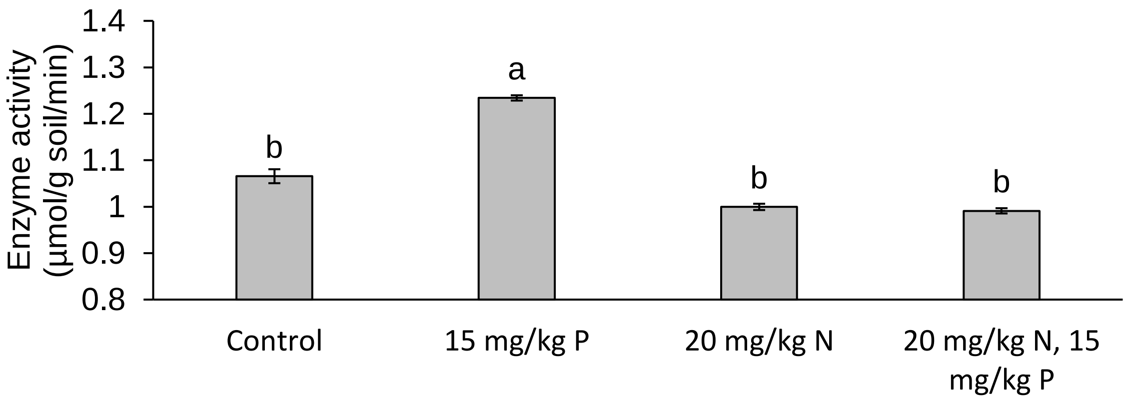

Figure 3.60. PK enzyme activity in rhizosphere soil in the control, 15 mg/kg P, 20 mg/kg P and 20 mg/kg N, 15 mg/kg P treatments.

3.3.5.3. Malate dehydrogenase and malic enzyme

P application did not have a significant effect on the activity of malate dehydrogenase (MDH), but the application of 15 mg/kg N in combination with 15 mg/kg P significantly (p=0.0145) reduced its activity from 1.95 – 2.12 in the control and 15 mg/kg P treatments to 1.78 µmol/g soil/min (Figure 3.61). This may be due to N application increasing microbial activity and thus its requirement for P. MDH activity an indication of the levels of malate synthesis from PEP-derived organic acids (Thuynsma et al., 2014 ab). The exudation of malate can be used to complex Al^{3+} in aluminium-phosphate compounds in the soil and make P more soluble under conditions of P deficiency (Thuynsma et al., 2014 ab).

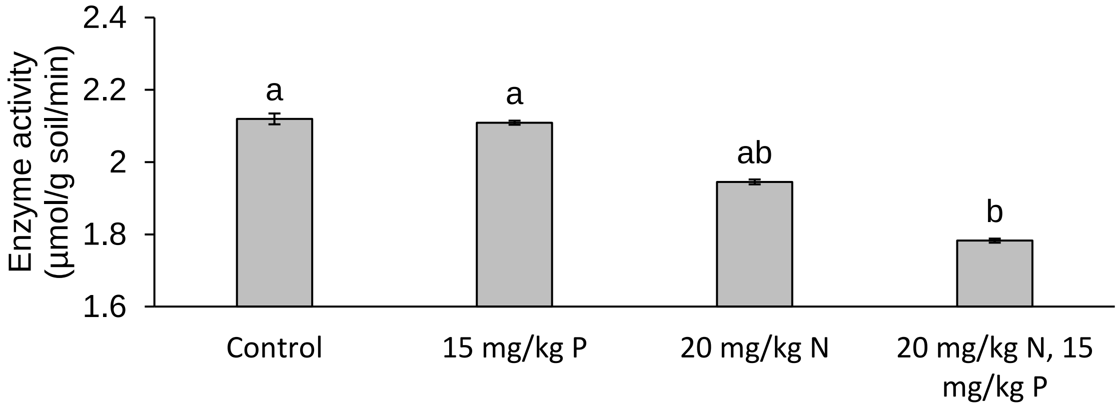

Figure 3.61. MDH enzyme activity in rhizosphere soil in the control, 15 mg/kg P, 20 mg/kg P and 20 mg/kg N, 15 mg/kg P treatments.

Malic enzyme (ME) activity was also significantly affected by N application (p=0.0359) but not by the application of P. The application of 20 mg/kg P with or without 15 mg/kg P reduced the activity of this enzyme to 0.0089 – 0.0085 µmol/g soil/min compared to its activity at 15 mg/kg P (0.031 µmol/g soil/min) (Figure 3.62). Malate produced by MDH can be transformed to pyruvate if not used for respiration or exudation for P uptake or it can be transformed to pyruvate via ME (Thuynsma et al., 2014ab).

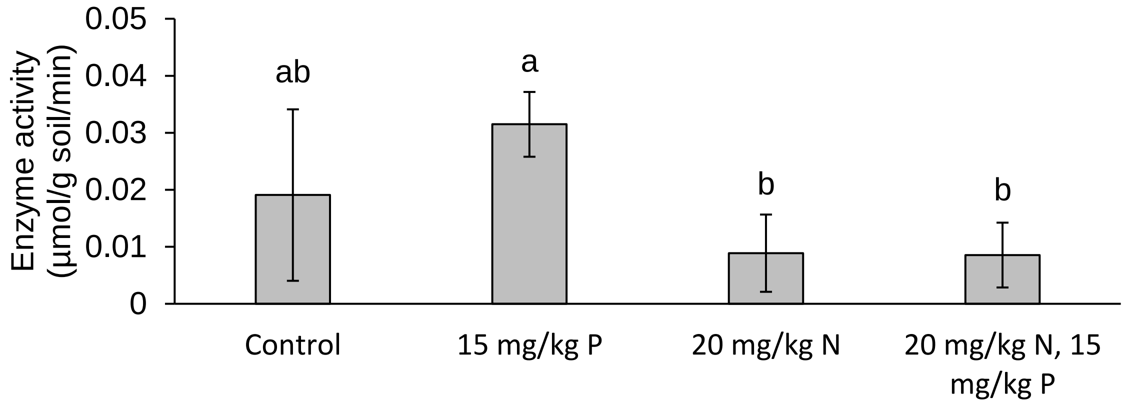

Figure 3.62. ME enzyme activity in rhizosphere soil in the control, 15 mg/kg P, 20 mg/kg P and 20 mg/kg N, 15 mg/kg P treatments.

3.4. Conclusions

N application at low rates (20 mg/kg) resulted in an increase in above-ground biomass without significantly reducing plant survival. The application of P fertilisers is not recommended in the year of establishment of a rooibos plantation as plant survival was significantly decreased by all rates of P application in this study and negatively correlated with soil P (R^2 = 0.6676) and foliar P (R^2 = 0.4320). No interactive effect between N and P on biomass response was found, and N application could not help rooibos to overcome P-toxicity contrary to previously published studies. Survival decreased significantly from 46.8 % in the control to 32.64% at the lowest P application of 15 mg/kg. A further decrease to 15.4 – 17.9 % occurred at P application of 45 – 60 mg/kg. Foliar P was also linearly correlated (R^2 = 0.527) with plant-available P at 20 – 40 cm, confirming that rooibos is unable to regulate its P uptake. An increase in Foliar P concentration of 0.06 to 0.10 % was associated with a reduction in plant survival to approximately 20 %. Furthermore, a significant negative correlation was observed between survival-adjusted yield and P application (R^2 = 0.8178) and plant-available P at 20 – 40 cm (R^2 = 0.6069). The application of 30 mg/kg P, for example, decreased survival-adjusted yield by nearly 60%, from 319 kg/ha to 135 kg/ha. These strong responses may also be due to unusually low rainfall (110 mm between June 2016 and June 2017, compared to the long-term mean annual rainfall of 206 mm) during this trial resulting in little dilution or uptake of the mineral fertilisers applied in the rooting zone of the seedlings.

K fertilisation however, had no negative effect on survival at all application rates studied, and at moderate application rates of 20 – 40 mg/kg, tended to increase yield from 333 kg/ha in the control to 455 – 597 kg/ha. The highest yield in this study (597 kg/ha), nearly double that of the unfertilised control, was obtained with the application 20 mg/kg K without the addition of N and P. This may be due to an enhanced resistance to drought, as K plays a role in lowering osmotic potentials in plants, improving stomatal regulation and water uptake by roots.

It is recommended that only low amounts of N (up to 20 mg/kg) and moderate amounts of KCl (20 – 60 mg/kg) are applied in the first year of a rooibos cultivation cycle to increase plant biomass response. Further field trials are recommended to study the effects of NPK fertilisation on established plants at least one year old as it is likely that rooibos plants will show a positive response to applied P once established and have larger root systems. Research is also needed on the effect of yield increase due to KCl application on the longevity of the rooibos plant, soil-water relations and its effects on tea quality.

CHAPTER 4 – Soil solute movement

4.1. Introduction

Leaching in soils is defined as the downward movement of soil solutes through a soil profile (Lehmann & Schroth, 2003). Leaching losses are particularly great when large amounts of inorganic nutrients are applied to well-drained, coarse sandy soils and may contribute to soil acidification and contamination of groundwater (Havlin, 1999). The downward movement of soil solutes requires a water input in excess of evapotranspiration and soil water-holding capacity, and is thus more common in humid climates, or during periods of heavy rainfall or irrigation (Havlin, 1999). Plants can mitigate this effect through nutrient cycling but the leaching of nutrients below the rooting depth can result in the permanent loss of the nutrients from the soil profile. The rooibos plant has a high density of fine feeder roots in the upper 25 cm of soil but takes up nutrients from deeper in the soil to a much lower extent (Matimati et al., 2014). Nitrogen in the form of nitrate is particularly susceptible to leaching due to its negative charge and its movement may cause the leaching of basic cations, since soil solutions must remain electrically neutral (Lehmann & Schroth, 2003). Phosphate is considered to be the most immobile of nutrients in the soil due to adsorption and precipitation processes. However, losses may be noteworthy in coarse soils or those high in organic matter (Lehmann & Schroth, 2003). The mobility of K is high in sandy soils under high rainfall conditions (Havlin, 1999). The aim of this study was to examine the distribution of the macronutrients N, P and K over depth in control and NPK fertilised treatments in order to determine the movement of applied nutrients between planting in June and the end of the rainfall season in October. Soil parameters examined were pH, EC, mineral ammonia and nitrate, and Bray II extractable P and K. Analysis of the NPK content of the seedlings at planting in June and of plants in both treatments in October was done to determine the effect of fertilisation.

4.2. Methods and materials

4.2.1. Experimental design and sampling

The fertiliser treatments selected for this experiment were taken from the field trial (control and a moderate NPK application of 20 mg/kg N, 30 mg/kg P and 20 mg/kg K) on deep soil (>100 cm depth) (Section 4.1.1). The treatments were replicated four times. Sampling was intended to be done every four months after planting, but due to the low rainfall and sandy nature of the soil, it was not possible to sample to depth without contamination from collapsing soil. Uncontaminated samples could only be obtained in Oct 2016 while the entire soil profile was moist. Composite (4) soil samples were taken with an auger in October 2016 at 0.1 m increments to a depth of 0.7 m, 0.2 m from the base of a living rooibos plant and in the row. Soil samples were kept cool and extracted with 2 M KCl within 24 hours of sampling and then air-dried for subsequent analysis of pH, EC, mineral and Bray II extractable P and K. Six random plants from each replicate were dug up intact, divided at the soil line and air-dried.

4.2.2. Soil chemical analysis

4.2.2.1. Soil pH and EC

Soil pH was measured in distilled water and in a 1 M KCl solution in a 1:2.5 solid to liquid ratio (Rowell, 1994). Soil electrical conductivity was measured in a 1:2.5 water extract and converted to the equivalent for a saturated paste extract (Sonmez et al., 2008).

4.2.2.2. Mineral N

Merck Spectroquant® Nitrate (14773) and Ammonium (100683) test kits were used to quantify mineral NO_3^- and NH_4^+ using spectrophotometry in the 2M KCl soil extracts (Merck KGaA, 64271 Darmstadt, Germany).

4.2.2.3. Plant-available phosphorus and potassium

The Bray II method was used to determine plant-available P and exchangeable K (Kuo, 1996).

4.2.3. Plant analysis

The below- and above-ground rooibos biomass was sent to the Western Cape Department of Agriculture, Elsenburg, South Africa, for elemental analysis of N, P and K. N was determined by Kjeldahl digestion and P and K were determined by ashing, dissolution in acid and ICP analysis.

4.2.4. Statistical analysis

Univariate tests of significance were performed on each soil and nutrient parameter using STATISTICA 12 data analysis software. Least significant difference (LSD) tests were used to separate differences between treatment means.

4.3. Results and discussion

4.3.1. Soil chemical parameters

4.3.1.1. Soil pH

The application of NPK fertiliser had a significant effect on pH (H_2O) ($p<0.0001$) lowering it by approximately 0.2 pH units throughout the soil profile (Figure 4.1). The effect of depth approached significance ($p=0.0629$), and the pH at 20 – 40 cm (pH 5.4) in the fertilised treatment was significantly lower than the pH at 0 – 10 cm in the unfertilised control (pH 5.8) (Figure 4.1). The NPK fertiliser treatment also significantly lowered soil pH (KCl) in this study ($p=0.0044$). A more pronounced decrease in soil pH (KCl) was observed at 20 – 40 cm in the fertilised treatment, compared to the control. This decrease in soil pH can be attributed to the acidifying effect of the nitrification of ammonia (Brady & Weil, 2010). Since the initial hydrolysis of the applied urea generates alkalinity (Brady & Weil, 2010), this can account for the relatively small decrease in pH.

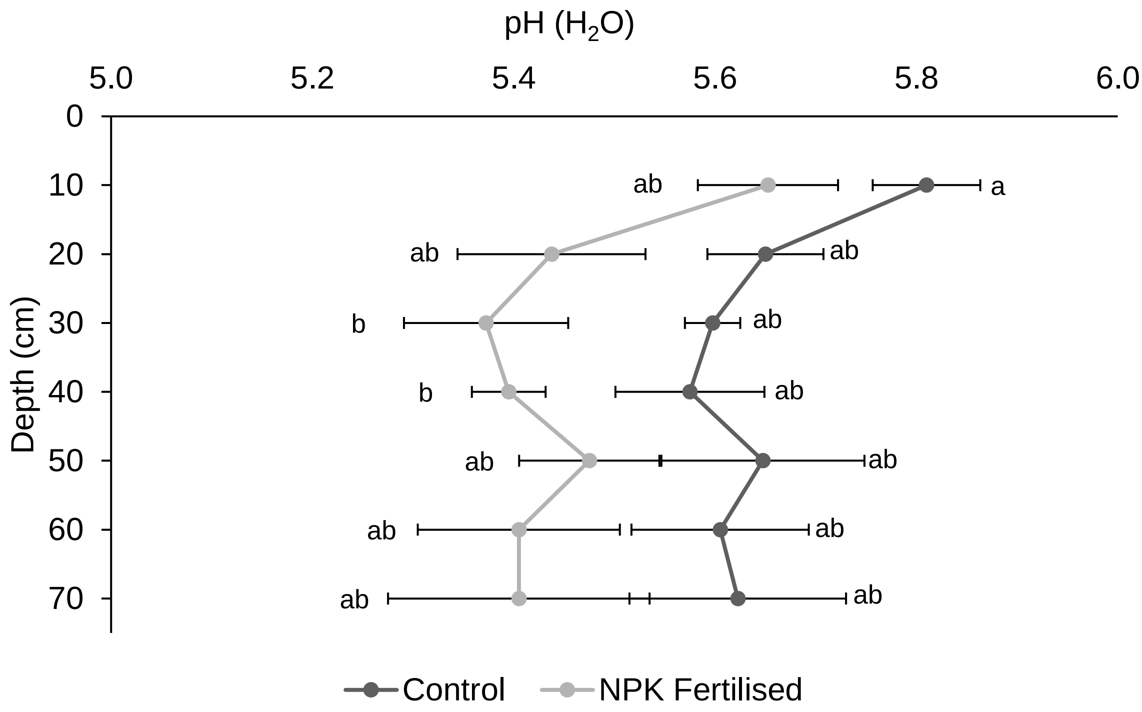

Figure 4.1. Vertical distribution (0 – 70 cm) of pH (H_2O) in unfertilized (control) and NPK fertilised (20 mg/kg N, 30 mg/kg P and 20 mg/kg K) deep soil treatments 4 months after planting.

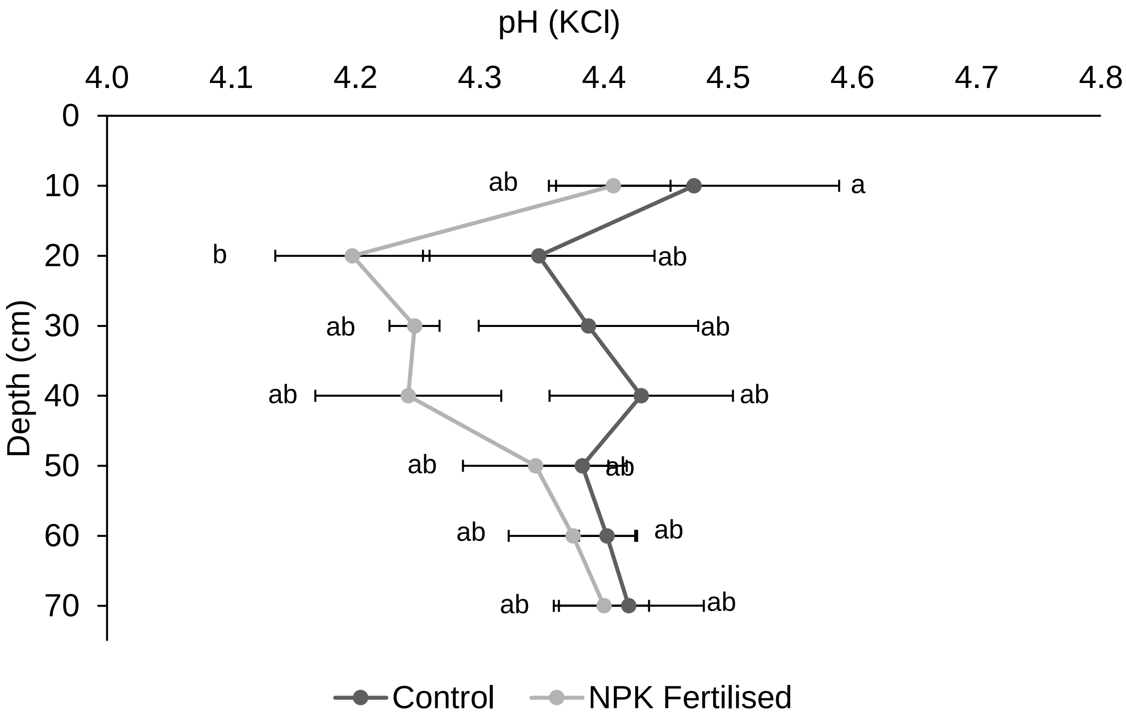

Figure 4.2. Vertical distribution (0 – 70 cm) of pH (1 M KCl) in unfertilized (control) and NPK fertilised (20 mg/kg N, 30 mg/kg P and 20 mg/kg K) deep soil treatments 4 months after planting.

4.3.1.2. Soil electrical conductivity.

NPK application was associated with a significant increase in soil EC throughout the soil profile ($p<0.0001$), although EC was generally low, with a maximum of 0.7 mS/m at 0 – 10 cm in the NPK fertilised treatment (Figure 4.3). EC also varied significantly according to depth ($p=0.0004$) and was higher at 0 – 10 cm and at 30 – 50 cm in the fertilised treatment, compared to the unfertilised control. EC values were raised by 0.06 – 0.1 mS/m at these depths, indicating a relative accumulation of mineral fertiliser and acidity. This bimodal distribution can be explained by the split fertiliser application, the second surface application in August 2016 accounting for the increase at the soil surface (0 – 10 cm) and the initial incorporated application causing the EC increase from 30 – 70 cm (Figure 4.3).

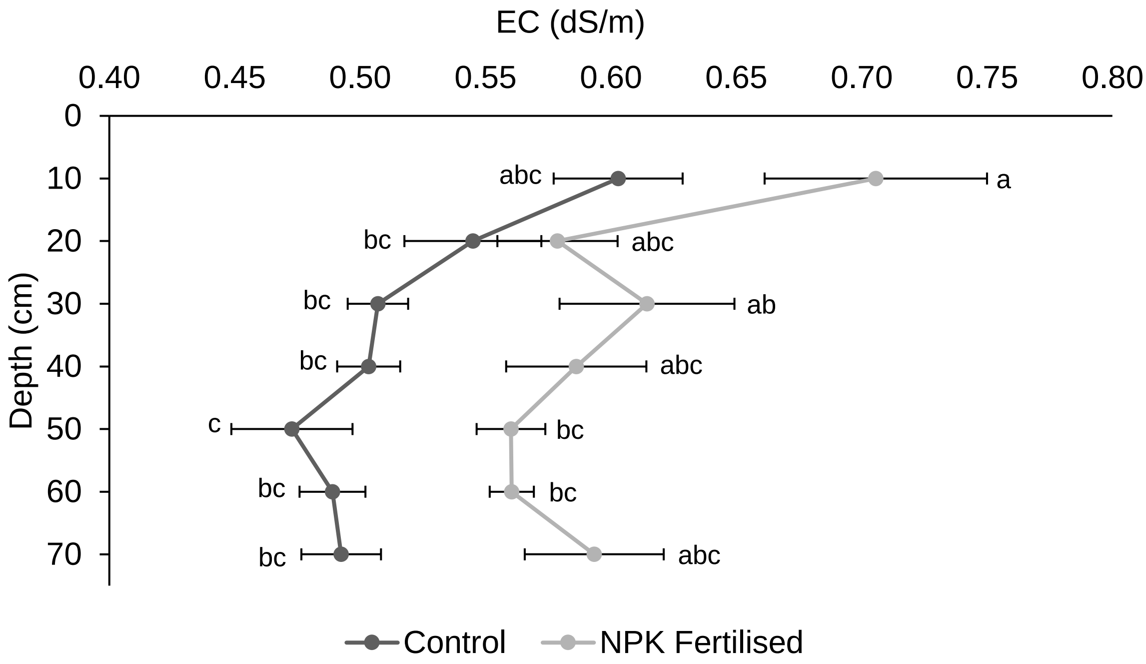

Figure 4.3. Vertical distribution (0 – 70 cm) of EC (dS/m) in unfertilized (control) and NPK fertilised (20 mg/kg N, 30 mg/kg P and 20 mg/kg K) deep soil treatments 4 months after planting.

4.5.1.3. Mineral N

Both ammonium (p=0.0014) and nitrate (p<0.0001) levels in the study were significantly increased by NPK fertilisation. Ammonium concentration in the fertilised treatment at 0 – 10 cm was 12.7 mg/kg, more than twice that of the unfertilised control (6.11 mg/kg). This can be ascribed to the topdressing of 10 mg/kg N applied per 20 cm depth in August 2016, little of which seems to have undergone nitrification or leaching deeper into the soil profile. A secondary zone of accumulation was observed at 30 – 60 cm, likely due to the initial fertilisation and incorporation to 20 cm of urea fertiliser applied in June 2016, followed by its leaching beyond this depth. A statistically significant interaction between treatment and depth was found (p=0.0391). Ammonium levels were approximately 10 times higher than nitrate levels (Figure 4.4 and Figure 4.5), indicating that little nitrification has occurred in the soil. This could be due to relatively low pH values (pH (KCl) < 5) as the nitrification process proceeds optimally at a neutral pH (Brady & Weil, 2010). Nitrate, is also more mobile in soils as it does not interact with the negatively charged surfaces in soils (Brady & Weil, 2010) and is thus less likely to accumulate in the soil profile. This may explain the higher NO_3^- levels observed from 0 – 40 cm in the fertilised treatment compared to the control and the insignificant effect of depth (p=0.5218) on nitrate distribution, indicating the movement and dilution of nitrate over depth, deeper than the original incorporation depth of the N fertiliser (Figure 4.5).

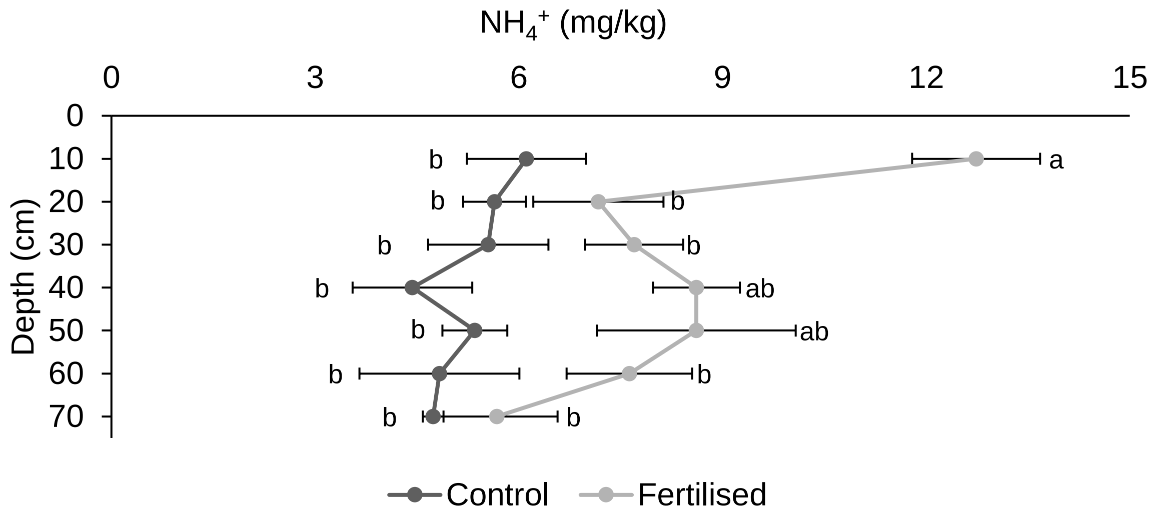

Figure 4.4. Vertical distribution (0 – 70 cm) of mineral NH_4^+ in unfertilized (control) and NPK fertilised (20 mg/kg N, 30 mg/kg P and 20 mg/kg K) deep soil treatments 4 months after planting.

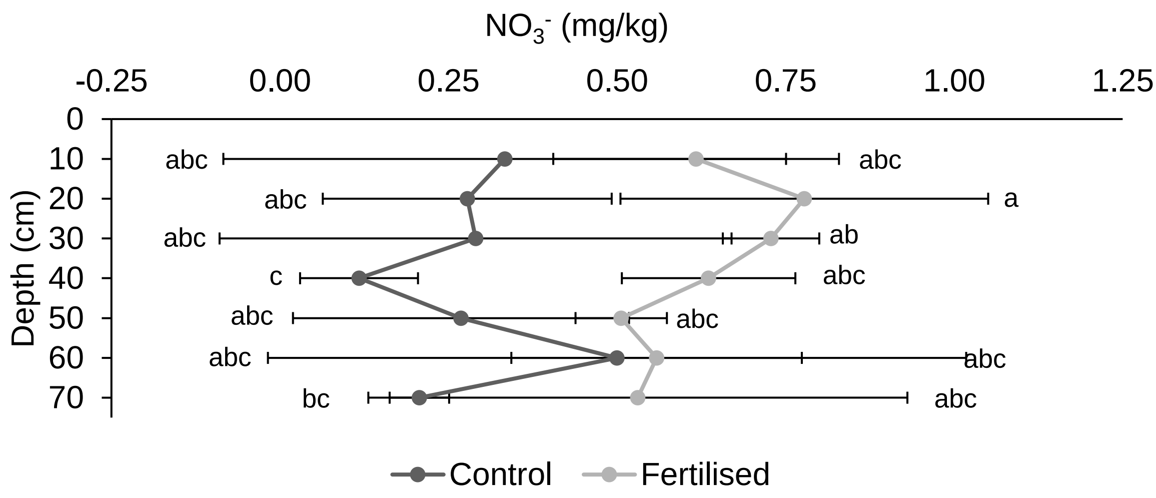

Figure 4.5. Vertical distribution (0 – 70 cm) of mineral NO_3^- in unfertilized (control) and NPK fertilised (20 mg/kg N, 30 mg/kg P and 20 mg/kg K) deep soil treatments 4 months after planting.

4.5.1.4. Plant-available P

The application of NPK did not have a significant effect on P distribution (p=0.1189) but a clear increase can be observed in the fertilised treatment at 20 – 30 cm in comparison to the control (Figure 4.6). This distribution can be attributed to the original depth of incorporation of all the P fertiliser at planting in June 2016. Phosphorus reacts readily with Al- and Fe- compounds in acid soils to form insoluble compounds with limited mobility (Brady & Weil, 2010), which can account for the smaller difference between the treatments deeper than 30 cm. The effect of depth on P distribution also falls just short of significance (p=0.0716), as well as the interactive effect between treatment and depth (p=0.0619). Leaching of P in sandy soils may be lower than in clay soils due to the lack macropores which facilitate drainage and removal of applied P (Liu et al., 2012).

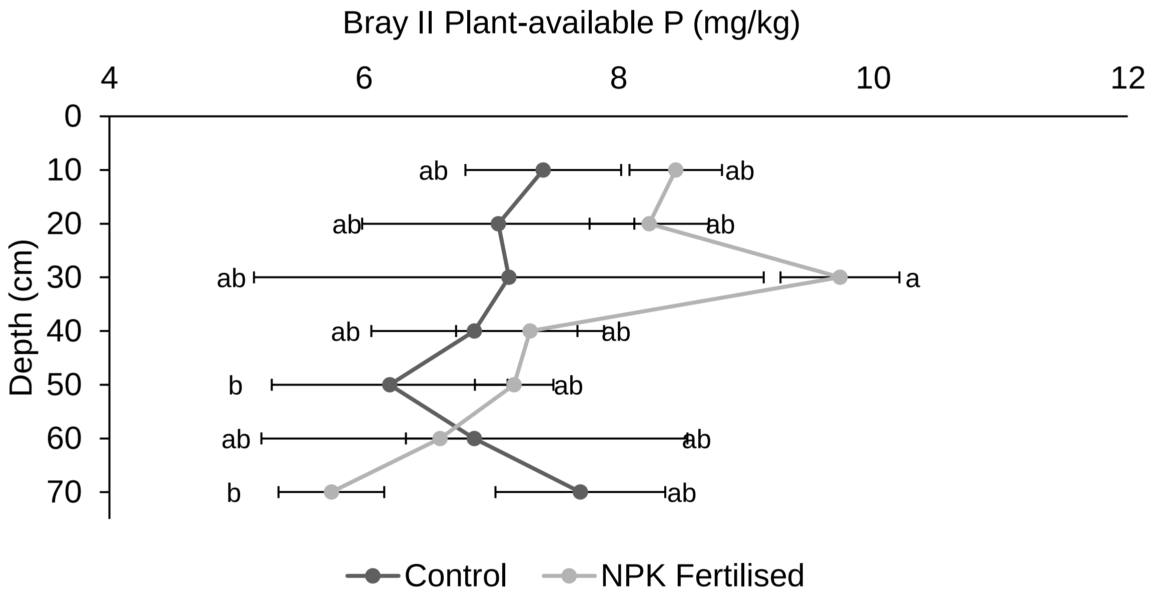

Figure 4.6. Vertical distribution (0 – 70 cm) of Bray II plant-available P in unfertilized (control) and NPK fertilised (20 mg/kg N, 30 mg/kg P and 20 mg/kg K) 4 months after planting.

4.5.1.5. Potassium

The application of NPK did not have a statistically significant effect on K distribution (p=0.6815), although somewhat higher levels were observed at 0 – 20 cm in the fertilised treatment (Figure 4.7). This is likely due to the top dressing applied in August 2016. No evidence was seen of the incorporated K applied at planting. This may be due to the K fertiliser having little relative effect due to relatively high K background levels (18.8 – 24 .3 mg/kg at 0 – 20 cm) compared to the application rate. The distribution of K according to depth was highly significant (p=0.0007). K tends to decline from 25.3 mg/kg at 0 – 10 cm to 17.8 mg/kg at 60 – 70 cm (Figure 4.7).

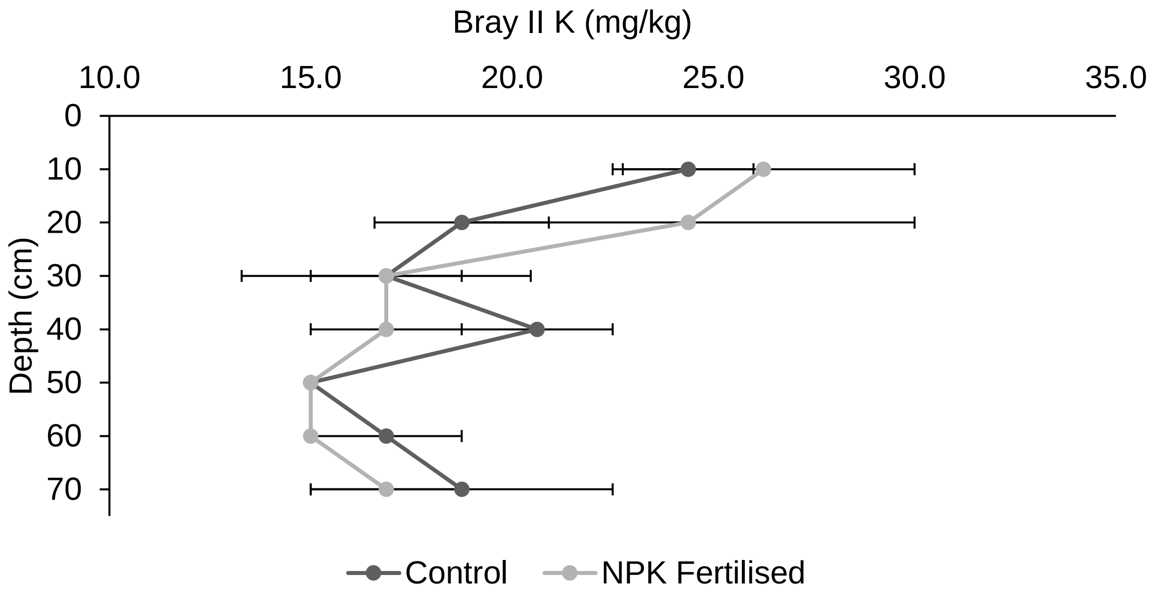

Figure 4.7. Vertical distribution (0 – 70 cm) of Bray II extractable K in unfertilized (control) and NPK fertilised (20 mg/kg N, 30 mg/kg P and 20 mg/kg K) deep soil treatments 4 months after planting.

4.3.2. Nutrient uptake

Shoot N content in the fertilised treatment (2.38 %) was significantly higher than that in the control (1.92 %) (Figure 4.8). This is likely due to increased uptake due to the higher N supply. Shoot N in both treatments was significantly higher than root N content. Root N was slightly higher in the fertilised (1.43 %) than in the control treatment (1.21 %), although the difference was not significant. Root N in both the control and fertilised treatment was lower than in the seedling in June (1.77 %) indicating a possible dilution effect.

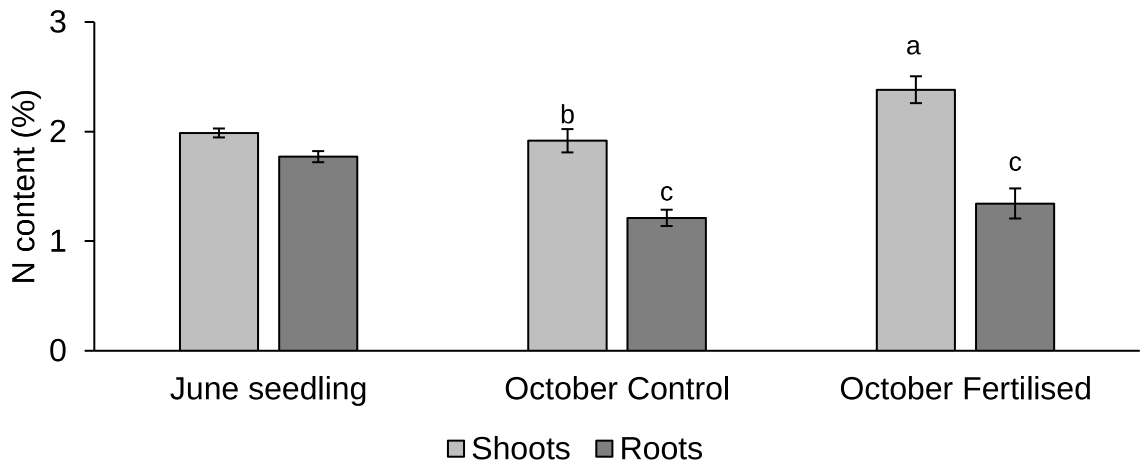

Figure 4.8. The average N content of the rooibos shoots and roots of the seedlings at planting (June 2016), and unfertilized (control) and fertilized (20 mg/kg N, 30 mg/kg P and 20 mg/kg K) deep site treatments 4 months after planting.

The root P content (1.32 %) of the fertilised treatment was almost 10 times higher than that of the control treatment (0.14 %) (Figure 4.9). Shoot P (0.44 %) in the fertilised treatment was also higher than that of the control treatment (0.12 %) although the difference was not statistically significant. Shoot and root P content in the fertilised treatment was also much higher than that in the shoot (0.15 %) and root P (0.59 %) of the seedlings planted in June, but these values could not be compared statistically. The control treatment root P content was less than a quarter of the root P content of the seedlings planted in June. This could be due to the seedlings being taken from a nursery in which P-containing fertiliser was applied. A dilution effect can account for the decrease in the unfertilised control shoot and root P content compared to the seedlings.

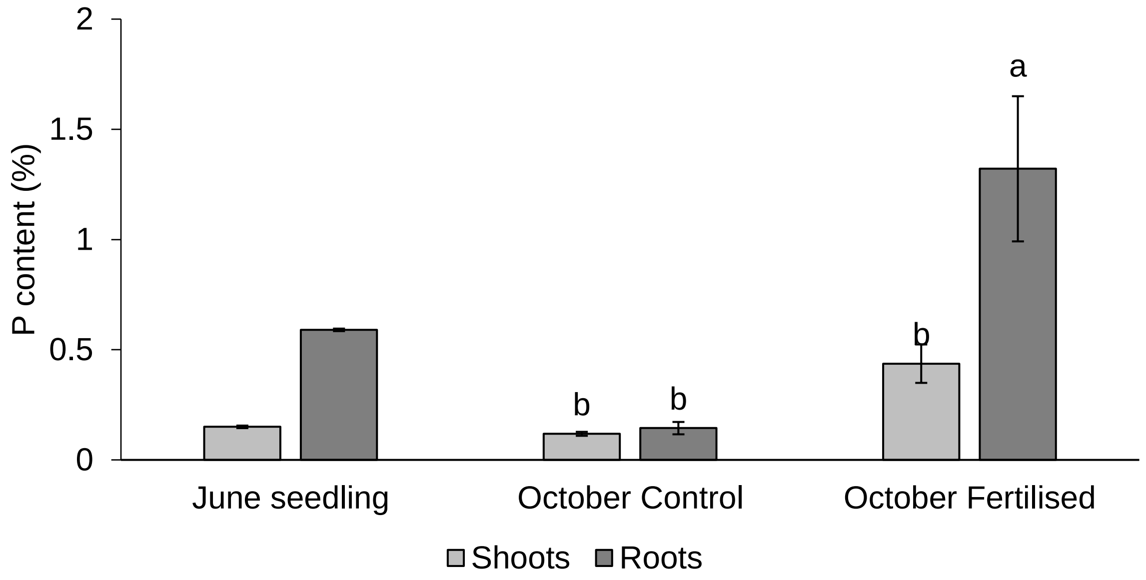

Figure 4.9. The average P content of the rooibos shoots and roots of the seedlings at planting (June 2016), and unfertilized (control) and fertilized (20 mg/kg N, 30 mg/kg P and 20 mg/kg K) deep site treatments 4 months after planting.

Similarly to P, root K content in the fertilised treatment (3.8 %) was significantly higher (more than four times) compared to the control (1.53 %) and higher than that of the seedlings planted in June (1.10%) (Figure 4.10). Shoot K in the fertilised treatment (1.53 %) was clearly higher than that of the control (1.10 %) and the June seedling (0.88 %), even though the difference was not statistically significant. A dilution effect is the likely cause for a decrease in root K content in the control compared to that in the seedling planted in June. It is likely that in October the rooibos plants in this study were still nutrient-accumulating phase and were storing P and K in their roots which would be used later in the season during periods of lower water availability and thus nutrient uptake (Strassen, 1987).

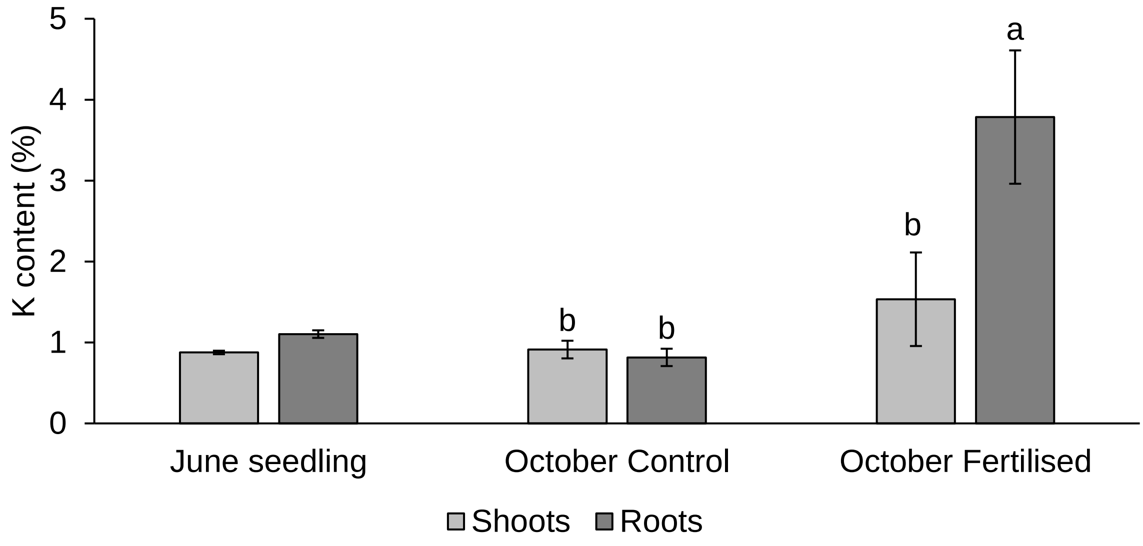

Figure 4.10. The average K content of the rooibos shoots and roots of the seedlings at planting (June 2016), and unfertilized (control) and fertilized (20 mg/kg N, 30 mg/kg P and 20 mg/kg K) deep site treatments 4 months after planting.

4.4. Conclusions

The results of this study indicate that little movement of applied P occurred, while some redistribution of incorporated ammonium occurred, modifying the distribution of pH and EC. The split application of top-dressed N and K applied in August 2016 seems to have remained at 0 – 10 cm, indicating that rainfall between this date and sampling in October 2016 was insufficient to cause much downward movement of these nutrients. Ammonium levels were much higher than nitrate throughout the soil profile, which indicated that nitrification was suppressed likely due to the low pH. Fertilised and unfertilised rooibos plants tended to accumulate more N in their shoot than in their roots in October, while P and K tended to accumulate more in the roots than in the shoots, but only in the fertilised treatment. This could indicate that these latter nutrients were more limiting than N for the growth of this leguminous species when the plant samples were taken and that P and K were being stored in the roots for later use (Strassen, 1987). P in particular is thought to accumulate in the vacuoles of this plant (Maistry et al., 2015), and the higher P levels may be indicative of the rooibos plants' inability to regulate uptake in the presence of higher amounts of nutrient, it being a plant adapted to low-P soils.

CHAPTER 5 – Lime incubation and pot trial

5.1. Introduction

Liming is a common agricultural practice in which the carbonates of calcium and magnesium are applied to the soil in order to increase pH, where soil acidity is a limiting factor for soil growth. These liming materials react with water and carbon dioxide to produce bicarbonates which in turn react with H^+ and Al^{3+}, neutralising their acidifying effect (Brady & Weil, 2010). Most crops grow optimally at pH 5.5 to 6.5, although some crops that originate from areas with highly leached and acidic soils such as blueberries, cranberries and many tropical crops tolerate levels as low as pH 4. Low pH suppresses plant growth indirectly by increasing the levels of soluble aluminium and magnesium to toxic concentrations, lowering the availability of the macronutrients N, P, K, Ca, Mg and S and the micronutrients B and Mo. Conversely, the availability of other micronutrients such as Fe, Zn and Cu may be high enough at low pH levels to cause toxicity (Brady & Weil, 2010). Low pH may also alter the composition and activity of soil microbial populations, such as N-fixing bacteria including *Bradyrhizobium*, which forms a symbiosis with rooibos tea plants (Hassen et al., 2012). Joubert et al. (1987) in their study of the nutrient requirements of rooibos observed that maximum growth was obtained by applying lime ($CaCO_3$) at half of the calculated rate obtained by using the following equation:

$$\text{Lime application (t/ha/15 cm)} \;=\; 1.7 + 3.9 \times \text{exchangeable acidity } (meq/100\ g)$$

The aim of this study was to perform a lime incubation to determine lime application rates to attain chosen pH levels in a soil typical of those in the rooibos producing region of Clanwilliam and to perform a two-month pot trial to observe the effect of lime application on pH, EC, exchangeable acidity and calcium and the biomass response of rooibos seedlings.

5.2. Methods and materials

5.2.1. Experimental design

Glass jars containing 50 g of air-dried, sieved (<2 mm) soil taken from the control treatment in the field trial at 0 - 20 cm were used to set up a lime incubation. A mass of laboratory-grade $CaCO_3$ equivalent to each of the four rates of lime application chosen (0, 0.25, 0.5, 1 and 2 t/ha, assuming a bulk density of 1 600 kg/m^3 and a depth of 15 cm) was added to respective jars, in duplicate. The contents of the jars were mixed well. Distilled water was added to the jars to wet the samples to field water capacity, the jars sealed with Parafilm® and the samples incubated for 10 days at 20°C, after which the samples were removed from the jars and air-dried for the chemical analysis of pH, EC and exchangeable acidity.

The intercept and slope of the linear regression equation obtained for the relationship between pH (KCl) and lime application was used (Figure 5.2) to calculate lime application rates (0, 0.5, 0.76, 1.03, 1.29 t/ha) to attain the pH (KCl) values of 5.0 (control), 5.5, 6.0, 6.5 and 7.0 for the subsequent pot trial. Laboratory grade $CaCO_3$ was mixed with dry sieved (<2 mm) soil of the same origin as that used for the lime incubation to attain the pH levels for each treatment. This was performed in triplicate. Two rooibos seedlings (sown in a nursery four months previously) were planted in each pot, and sufficient water was added to the soil to wet the soil to field water capacity. The pots were placed in a glasshouse at the Department of Agronomy, Stellenbosch University, South Africa on the 9th of June 2017 and kept at constant temperature of 20°C and grown for two months. The rooibos plants were destructively harvested on 3rd of August 2017 and air-dried. The biomass response was calculated as the average mass of the plants remaining at the end of the pot trial. The soil was removed from the pots and air-dried for chemical analysis of pH, exchangeable acidity and exchangeable Ca.

5.2.2. Soil chemical analysis

5.2.2.1. Soil pH and EC

Soil pH was measured in distilled water and in a 1M KCl solution in a 1:2.5 solid to liquid ratio (Rowell, 1994). Soil electrical conductivity was measured in a 1:2.5 water extract and converted to the equivalent for a saturated paste extract (Sonmez et al., 2008).

5.2.2.2. Exchangeable cations and acidity

Exchangeable Ca was determined via the 1 M ammonium acetate (pH 7.0) method, using the centrifuge procedure. Exchangeable acidity was measured using the 1 M KCl extraction method (Thomas, 1982)

5.2.3. Statistical analysis

Univariate tests of significance were performed on each parameter using STATISTICA 12 data analysis software. Least significant difference (LSD) tests were used to separate differences between treatment means in the pot trial, and Spearman and Pearson tests were used to determine correlations between soil parameters and biomass response.

5.3. Results and discussion

5.3.1. Lime incubation results

5.3.1.1. Soil pH (H_2O)

As illustrated in Figure 5.1, a linear relationship ($R^2 = 0.9785$) between soil pH (H_2O) and lime application was found within the lime application range. The effect of lime application on pH (H_2O) was highly significant ($p<0.0001$),

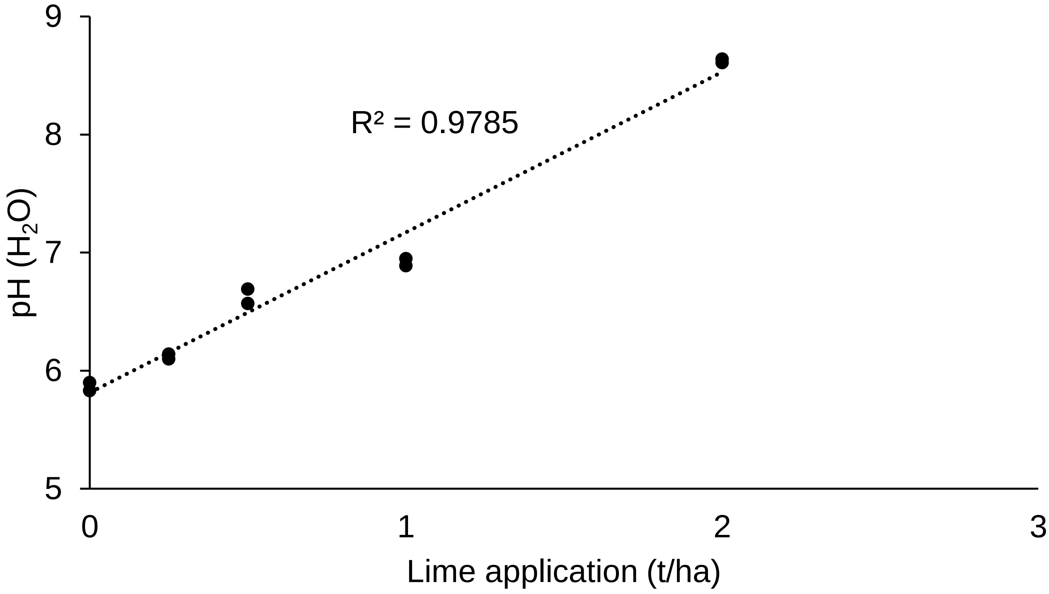

Figure 5.1. Correlation between pH (H_2O) and lime application.

5.3.1.2. Soil pH (KCl)

The application of lime had a highly significant effect ($p<0.0001$) on pH in KCl. The clear positive linear relationship ($R^2=0.9871$) between lime application and pH within this application range is illustrated in Figure 5.2. An increase in pH (KCl) of 1.9 units for each t/ha of lime applied was observed from the linear regression equation in Figure 5.2.

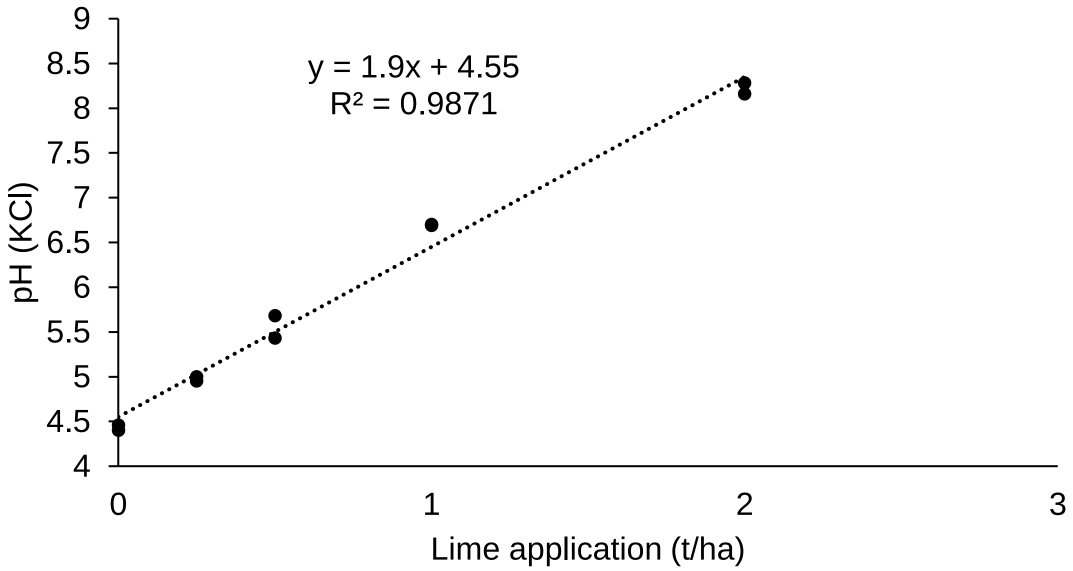

Figure 5.2. Correlation between pH (KCl) and lime application.

5.3.1.3. Electrical conductivity

The application of lime significantly ($p<0.0001$) increased EC from approximately 0.77 dS/m at applications between 0 and 1 t/ha to 3.8 dS/m. The non-linear positive correlation (R^2 = 0.8540) between lime application and EC is can be seen in Figure 5.3. This is likely due to the higher equivalent conductance of OH^- ions compared to basic cations such as Na+ and K+ (McBride, 1994).

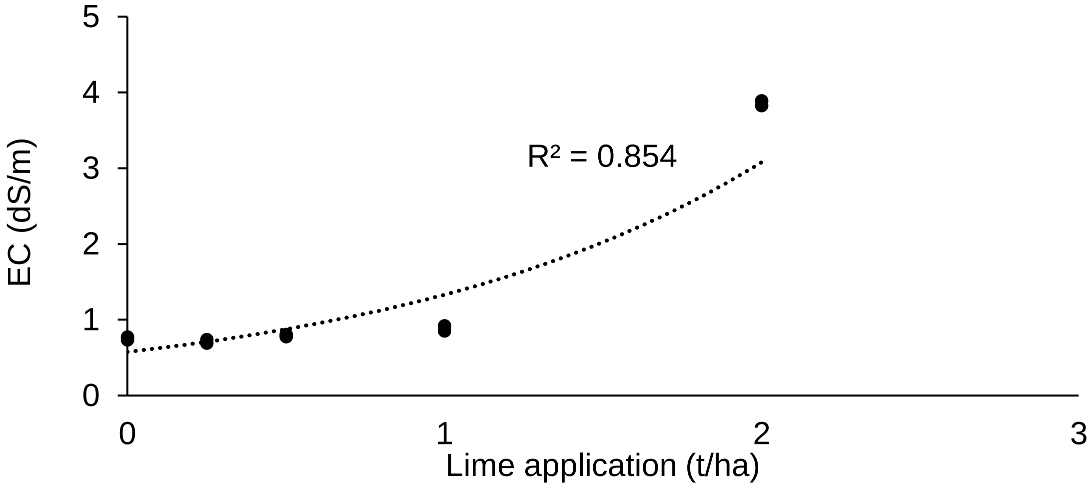

Figure 5.3. Correlation between EC and lime application.

5.3.1.4. Exchangeable acidity

Exchangeable acidity was also significantly ($p<0.0001$) affected by the application of lime. The application of rates higher than 0.25 t/ha resulted in a dramatic decrease in acidity from 0.15 cmolc/kg to 0.05 cmolc/kg at the highest application rates. This negative correlation (R^2 = 0.7884) is illustrated in Figure 5.4.

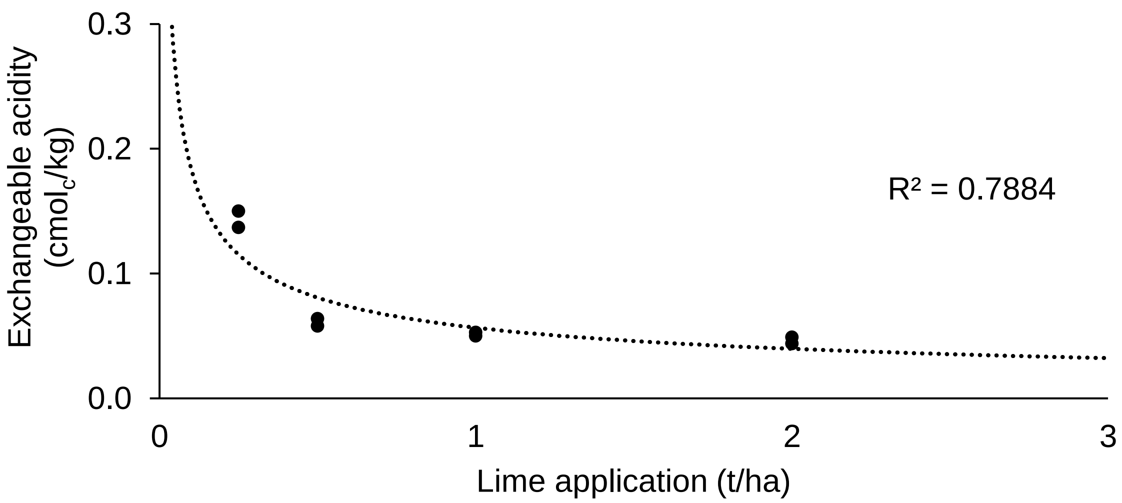

Figure 5.4. Correlation between exchangeable acidity and lime application.

5.3.2. Pot trial results

5.3.2.1. pH (KCl)

Lime application significantly increased pH (KCl) in the pot trial ($p<0.0001$). Soil pH increased from 5.16 in the control treatment to pH 8.20 at the highest lime application (1.29 t/ha) (Figure 5.5).

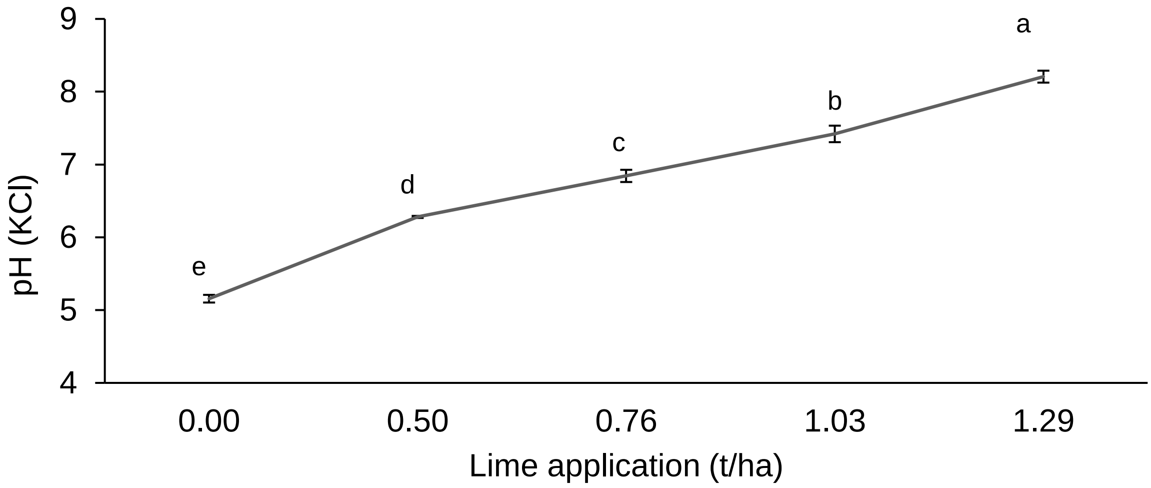

Figure 5.5. The effect of lime application on pH (KCl) in pot trial.

5.3.2.2. Exchangeable acidity

Exchangeable acidity was found to decrease significantly ($p=0.0002$) with the application of lime, decreasing from a maximum of 0.13 cmol$_c$/kg in the control to 0.05 – 0.07 cmol$_c$/kg at application rates between 0.5 and 1.29 T/ha. No significant decrease in acidity was observed at application rates higher than 0.5 t/ha (Figure 5.6). This is likely due to bicarbonate reacting with H^+ and Al^{3+}, which was replaced by Ca^{2+} on cation exchange sites in the soil (Brady & Weil, 2010).

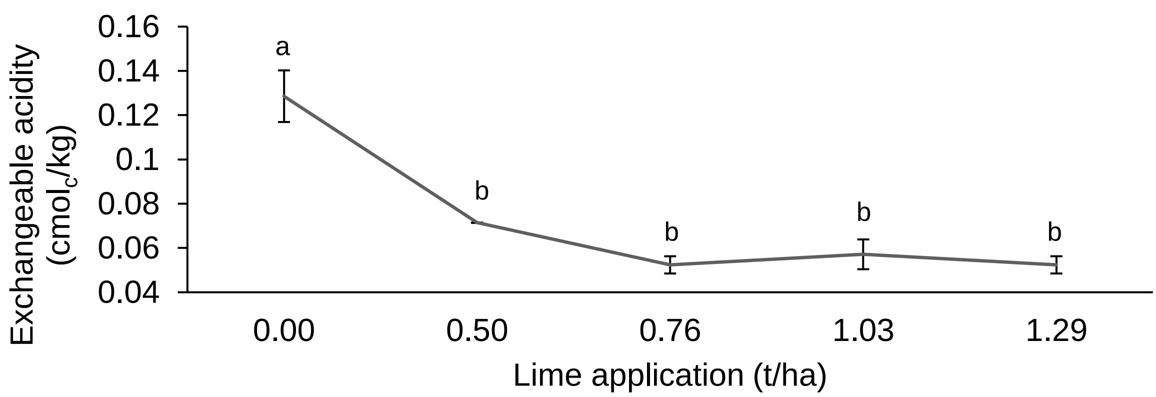

Figure 5.6. The effect of lime application on exchangeable acidity in the pot trial.

5.3.2.3. Exchangeable Ca

As can be expected, the decrease in acidity due to lime application was concomitant with a significant increase ($p<0.0001$) in levels of exchangeable calcium in the pot trial, which more than doubled from 0.43 $cmol_c$/kg in the control treatment to over 1.01 $cmol_c$/kg at lime application rates of 1.03 – 1.29 T/ha (Figure 5.7).

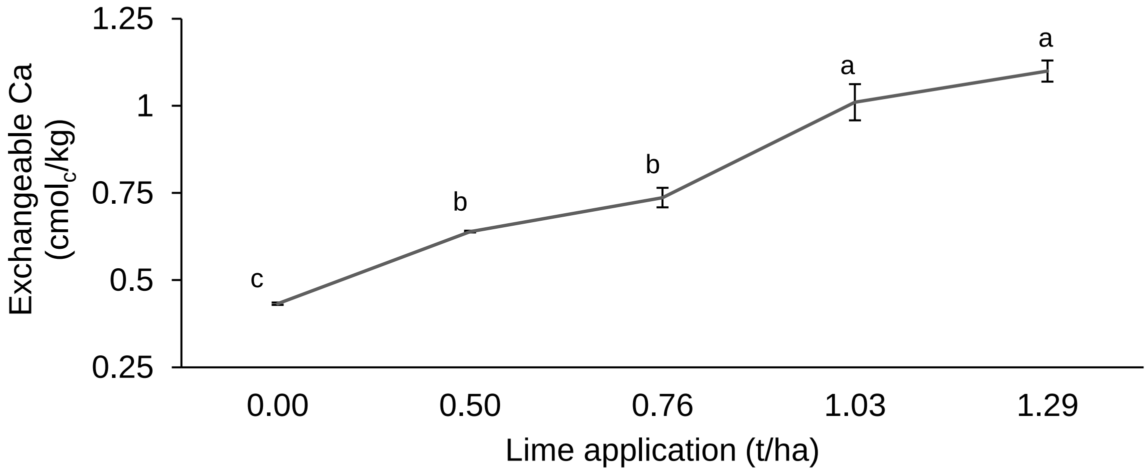

Figure 5.7. The effect of lime application on exchangeable Ca in the pot trial.

5.3.2.4. Seedling biomass

A significant increase ($p = 0.0124$) in seedling biomass was observed with the application of lime in this pot trial. In the control treatment, average seedling mass was 0.52 g/plant. This significant increased to a maximum of 0.86 – 1.01 g/plant at application rates of 0.76 – 1.29 t/ha (Figure 5.8). This corresponds to a pH range of 6.84 – 8.21 (Figure 5.5), exchangeable acidity of 0.5 – 0.6 $cmol_c$/kg (Figure 5.6) and exchangeable calcium levels of 0.74 – 1.10 $cmol_c$/kg (Figure 5.7).

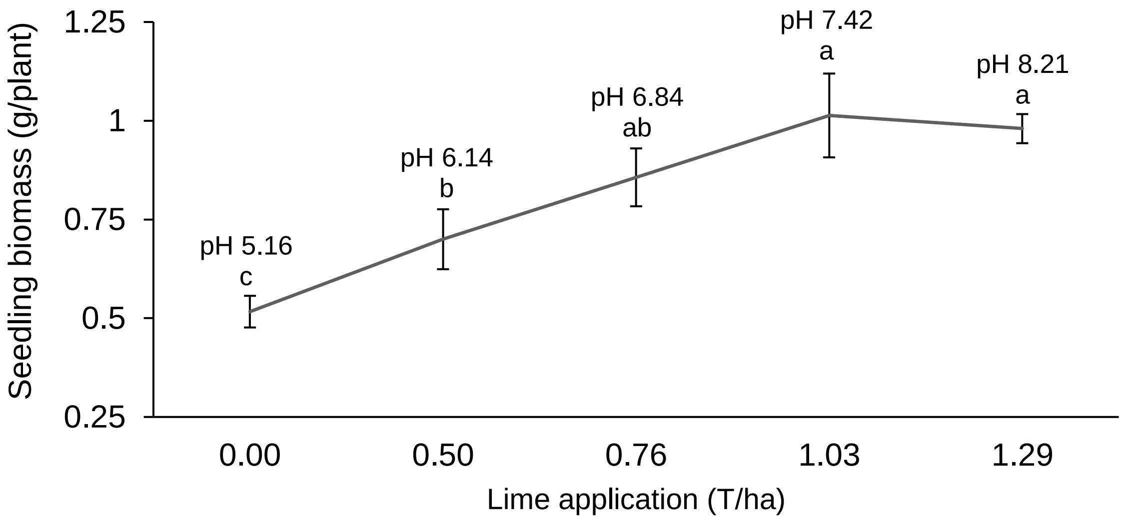

Figure 5.8. The effect of lime application on seedling biomass in the pot trial.

Figure 5.9 illustrates the dramatic effect that lime application had on the appearance of the rooibos seedlings. Seedlings in the control treatment are visibly stunted and chlorotic in comparison to those that received lime application. The seedlings that received the three highest applications (0.76, 1.03 and 1.29 t/ha) are larger and have a deeper green needle colour.

Figure 5.9. Photograph of rooibos seedlings in the liming pot trial after two months of growth. The control treatment is the row furthest to the left, and the highest lime application is on the right.

Table 5.1 displays the correlation values obtained for biomass versus soil chemical parameters using Pearson and Spearman tests. A highly significant correlation was observed for all parameters in the Pearson correlation test. All parameters except exchangeable acidity were positively correlated with seedling biomass (Table 5.1). The highest correlation coefficient obtained was for exchangeable Ca (+0.83). The pH (KCl) and exchangeable acidity had correlation coefficients of +0.77 and –0.68 respectively (Table 5.1).

Table 5.1 Correlations between seedling biomass and soil parameters using Pearson and Spearman correlation tests.

	Pearson		Spearman	
Parameter	Coefficient	P-value	Coefficient	P-value
pH (KCl)	+0.77	<0.01	+0.78	<0.01
Exchangeable acidity	–0.68	<0.01	–0.59	0.02
Exchangeable Ca	+0.83	<0.01	+0.83	<0.01

Figure 5.10 illustrates that seedling biomass was well correlated with (a) pH (KCl), (b) exchangeable acidity and (c) exchangeable Ca with R^2-values of 0.6140, 0.5655, and 0,6230 respectively.

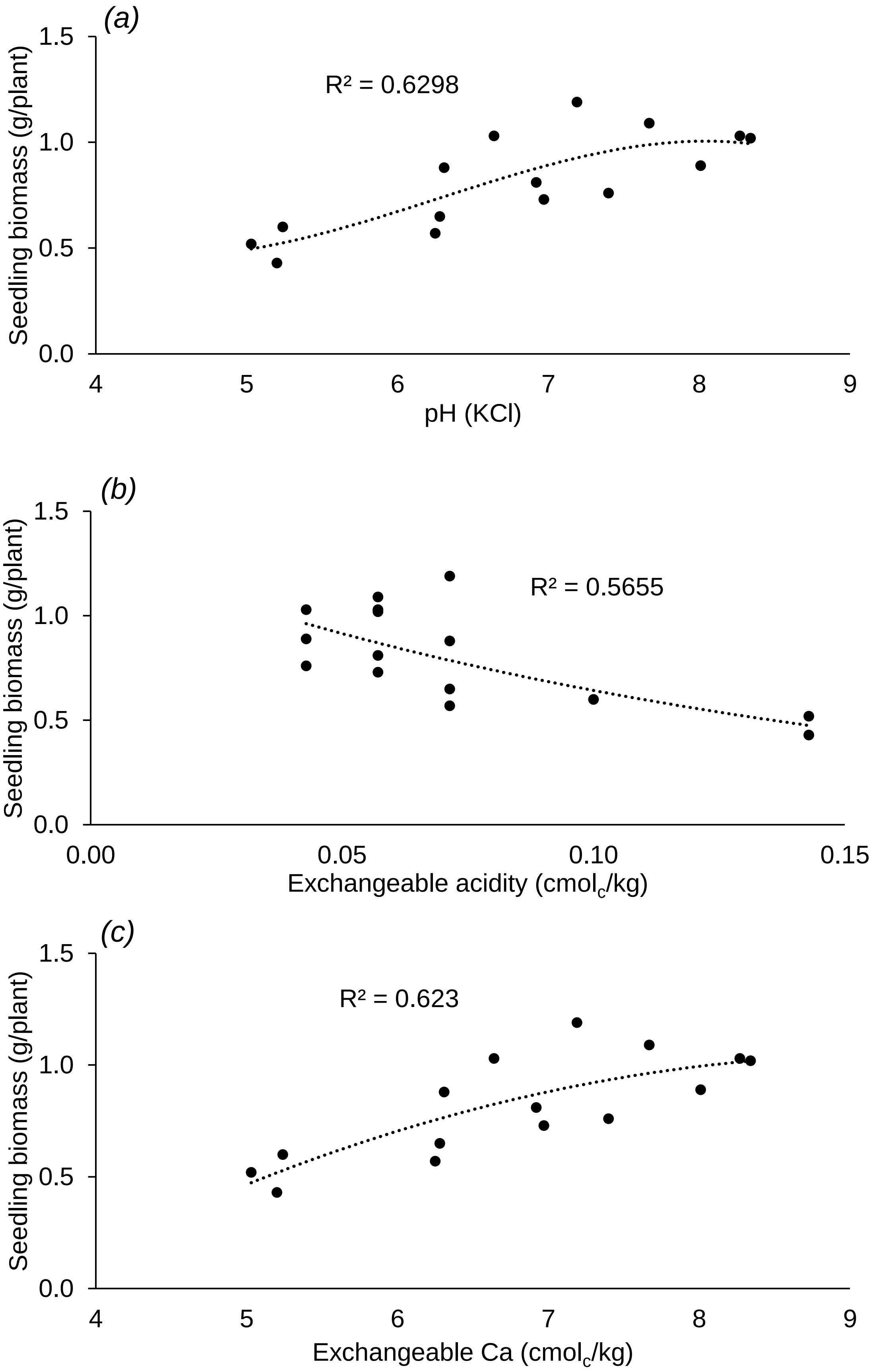

Figure 5.10. Correlation between seedling biomass and (a) pH (KCl), (b) exchangeable acidity and (c) exchangeable Ca.

5.4. Conclusions

Lime application was highly effective at increasing the pH and decreasing the exchangeable acidity of a soil typical of those on which rooibos is cultivated in the Clanwilliam region. In the pot trial the application of lime increased pH (KCl), decreased exchangeable acidity and increased exchangeable Ca. Lime application at all rates up to 1.29 t/ha positively influenced rooibos seedling biomass. Application rates of 1 – 1.3 t/ha nearly doubled the mass of rooibos seedlings after two months. The ideal pH for rooibos seedling growth in this study was found to be around pH (KCl) 7.4. This may be a chemical effect due to an increase in availability of macronutrients and micronutrients Mo and B, or the increase in pH and Ca levels in the soil increased the activity of symbiotic bacteria responsible for nitrogen fixation and helped the seedlings to overcome an N-deficiency. This later possibility can be addressed, as seedlings have been sent for total N and isotope analysis. The results were not yet available at the time of publishing. Further research on this topic is recommended to investigate the effects of lime grade and application rates on rooibos response in the field.

GENERAL CONCLUSIONS

The application of P fertilisers is not recommended in the year of establishment of a rooibos plantation as plant survival was significantly decreased by all rates of P application in this study and negatively correlated with soil P (R^2 = 0.6676) and foliar P (R^2 = 0.4320). No interactive effect between N and P on biomass response was found, and N application could not help rooibos to overcome P-toxicity, contrary to literature. Survival decreased significantly from 46.8 % in the control to 32.64% at the lowest P application of 15 mg/kg. A further decrease to 15.4 – 17.9 % occurred at P application of 45 – 60 mg/kg. Foliar P was also positively correlated (R^2 = 0.527) with plant-available P at 20 – 40 cm, confirming that rooibos is unable to regulate its P uptake. An increase in foliar P concentration of 0.06 to 0.10 % was associated with a reduction in plant survival to approximately 20 %. Furthermore, a significant negative correlation was observed between survival-adjusted yield and P application (R^2 = 0.8178) and plant-available P at 20 – 40 cm (R^2 = 0.6069). The application of 30 mg/kg P, for example, decreased survival-adjusted yield by nearly 60%, from 382.1 kg/ha to 156.7 kg/ha. These strong responses may be due to unusually low rainfall during this trial resulting in little dilution or uptake of the mineral fertilisers applied in the rooting zone of the seeds, since a positive height response to P application of 15 mg/kg and 30 mg/kg was observed at 8 and 4 months after planting, respectively. K fertilisation however, had no significant effect on survival, and at moderate application rates of 20 – 40 mg/kg, tended to increase yield from 333 kg/ha in the control to 455 – 597 kg/ha. The highest yield in this study (597 kg/ha), nearly double that of the unfertilised control was obtained with the application 20 mg/kg K without the addition of N and P. This may be due to an enhanced resistance to drought, as K plays a role in lowering osmotic potentials in plants, improving stomatal regulation and water uptake by roots. Further field trials are recommended to study the effects of NPK fertilisation on established plants at least one year old and applying only low amounts of N (20 mg/kg or less) and moderate amounts of KCl (20 – 60 mg/kg) at planting, on the response of rooibos from its second year of growth onward, since it is likely that rooibos plants will show a positive response to P application once established and have a larger root system. Research is also needed on the effect of yield increase due to KCl application on the longevity of the rooibos plant, soil-water relations and its effects on tea quality.

The results of the leaching study indicate that little movement of applied P occurred, while some redistribution of incorporated ammonium occurred, modifying the distribution of pH and EC. The split application of top-dressed N and K applied in August 2016 seems to have remained at 0 – 10 cm, indicating that rainfall between this date and sampling in October 2016 was insufficient to cause much downward movement of these nutrients. Ammonium levels were much higher than nitrate throughout the soil profile, which indicated that nitrification was suppressed likely due to the low pH of the soil. Fertilised and unfertilised rooibos plants tended to accumulate more N in their shoot than in their roots in October, while P and K tended to accumulate more in the roots than in the shoots, but only in the fertilised treatment. This could indicate that these latter nutrients were more limiting than N for the growth of this leguminous species when the plant samples were taken and that P and K were being stored in the roots.

Lime application at all rates up to 1.29 t/ha positively influenced rooibos seedling biomass. Lime application rates of 1 – 1.3 t/ha nearly doubled the mass of rooibos seedlings after two months, compared to the control. The ideal pH for rooibos seedling growth in this study was found to be around pH (KCl) 7.4. Further research on this topic is recommended to investigate the effects of lime grade and application rates on rooibos response in the field.

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